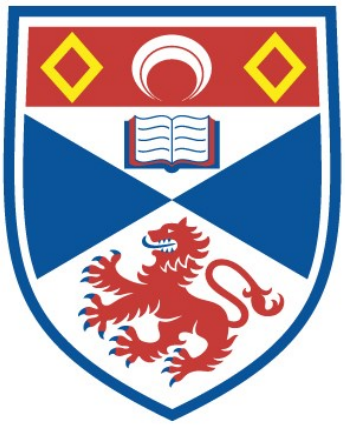
SOME ELECTROPHILIC REACTIONS OF PYRROLE AND RELATED COMPOUNDS

Robert Scott Alexander

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1977

SOME ELECTROPHILIC REACTIONS

OF

PYRROLE AND RELATED COMPOUNDS

A Thesis

presented for the degree of

DOCTOR OF PHILOSOPHY

in the Faculty of Science of the

University of St. Andrews

by

ROBERT SCOTT ALEXANDER, B.Sc.

St. Andrews

September 1977

to my parents

Abstract

Chapter One describes the mechanism of acid-catalysed hydrogen-exchange in methylpyrroles. Using buffer solutions it was found that this reaction is subject to general acid catalysis, confirming an A-S_E2 mechanism. It was also found that the rate of hydrogen-exchange at the β-position of pyrrole was similar to that at the α-position. This seems to conflict with the known preference for α-substituted products obtained under 'synthetic' experimental conditions. However, both these findings have been rationalised in terms of the electron densities and the localisation energies prevalent in the pyrrole ring.

The mechanism of the reaction between 4-dimethylamino-benzaldehyde (DMAB) and pyrroles in acid solution (Ehrlich's Reaction) is described in Chapter Two. This consists of a rate-determining electrophilic attack of O-protonated DMAB on unprotonated pyrrole followed by a rapid loss of a water molecule to give the highly-coloured conjugated product. The activating effect of methyl groups at various positions on the pyrrole ring on this reaction was also determined.

Acid-catalysed hydrogen-exchange in methylthiophens is the subject of Chapter Three. In contrast to the situation found in pyrroles, the rate of exchange at the β-position of thiophen is much slower than at the α-position. An explanation for this is found in the difference in electron densities found in the two ring systems. The activating effect of methyl groups at various positions

on the thiophen ring on the exchange reaction was also studied. It was found that these effects were not additive. The activating parameters for the exchange reactions at the α- and β-positions were also calculated and discussed.

In Chapter Four the practical difficulties encountered in the quantitative determination of the clinically important pyrroles porphobilinogen and cryptopyrrole are discussed. A series of reagents were used in an attempt to improve on the present colourimetric test employing DMAB. It was found that the measurement of cryptopyrrole under acidic conditions is straightforward using DMAB, 2,4,6-trimethoxybenzaldehyde (TMB) or 2,4-bis(dimethylamino)benzaldehyde (BDMAB). In contrast to this situation, however, none of the reagents tested gave a completely stable coloured solution when reacted with aqueous porphobilinogen solutions. A test was devised, however, using the BDMAB reagent in acid solution which gave a colour, stable for up to 40 minutes, when reacted with test porphobilinogen solutions.

DECLARATION

I declare that this thesis is my own composition, that it is based on the results of experiments carried out by me, and that it has not previously been presented for a Higher Degree.

This thesis describes results of research carried out in the Department of Chemistry of the United College of St. Salvator and St. Leonard, University of St. Andrews, under the supervision of Dr. A.R. Butler, between October 1974 and June 1977.

CERTIFICATE

I hereby certify that Robert S. Alexander has spent twelve terms of research work under my supervision, has fulfilled the conditions of Ordinance General No. 12 and Resolution of the University Court 1967, No. 1, and is qualified to submit the accompanying thesis in application for the degree of Doctor of Philosophy.

Director of Research

ACKNOWLEDGEMENTS

I would like to thank Dr. A.R. Butler for his help and encouragement in all aspects of this work.

My thanks go to the members of the Chemistry Department who have given assistance, in particular Mr. P. Pogorzelec for his advice on pyrrole syntheses. I am grateful to Mrs. W. Pogorzelec for typing this thesis.

Finally, I gratefully acknowledge a studentship from the Science Research Council.

CONTENTS

'The wily lunatic is lost if through the narrowest crack he allows a sane eye to peer into his locked universe and thus profane it.'

Colette, 'Freedom', Earthy Paradise (1966), 2, ed. Robert Phelps.

General introduction to pyrrole chemistry

The chemistry of pyrroles is rich in substitution reactions which can readily be recognised to involve attack by an electrophilic reagent. On the other hand, little is known of the reactions of pyrrole with radicals or with nucleophilic reagents. With regard to electrophilic substitution, however, although the general character of these reactions is usually apparent, very few detailed mechanistic studies have been made.

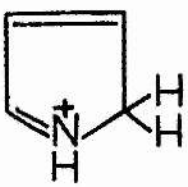
The distinct preference for these compounds to undergo substitution rather than addition reactions indicates the degree of aromaticity associated with their structure; this aromaticity being attributed to the delocalisation into the ring of the 'lone-pair' electrons on the nitrogen atom. It should be noted, however, that Diels-Alder adducts have been postulated as possible intermediates in some of their reactions (1, 2, 3) and that N-methyl pyrrole undergoes one such reaction with dimethylacetonedicarboxylate (2). Both pyrrole and its 1-phenyl derivative can also be reduced by zinc and acetic acid to yield the Δ^3 pyrrolines.

As mentioned above, the reactivity of pyrroles towards electrophilic reagents is high and in this respect they are often compared with phenols. This reactivity is modified in the expected way by substituents and, in general the α-positions are more reactive than the β-positions, but the difference is not so great that it cannot be outweighed by electron-attracting substituents. A few examples are given to illustrate these points. Halogenation of the pyrrole ring occurs very readily and invariably leads to polysubstituted products, the tetra-substituted product being the

only easily isolable product (4, 5, 6, 7, 8). Extensive studies of the coupling abilities of pyrroles with a range of diazonium salts of widely differing electrophilic activities have been carried out by Treibs and Fritz (9) and Butler, Shepherd and Pogorzelec (68). The pyrroles involved vary from highly activated alkyl-substituted compounds to highly deactivated ring systems containing nitro- and ester groupings. The results from these studies clearly demonstrate that substituents modify the pyrrole ring in a way that is predictable in terms of their electron donating or withdrawing properties. Strongly electron-withdrawing groups attached to an α-position of the pyrrole ring can reverse the relative reactivities of the remaining α- and β-positions. Thus, nitration of 2-nitropyrrole leads to the formation of 2, 4- and 2, 5-dinitropyrrole in the ratio 4:1 (10) and nitration of 2-formylpyrrole leads to equal quantities of the 4- and 5-nitro products (11).

The weakly acidic properties of pyrroles are used in the preparation of N-substituted compounds, generally via an N-alkali metal salt. These alkali metal salts, prepared by reaction of pyrrole and alkali metals in liquid ammonia, can be treated with alkyl halides, acyl halides or ethyl chloroformate to yield the corresponding N-alkyl, N-acyl or N-ester compound.

In the case of nucleophilic substitution in the pyrrole ring very little is known and in the few cases which appear to fall into this category (addition of bisulphite (12) and attack by hydroxylamine (13)) the mechanism is still in doubt. The situation as regards radical substitution is no clearer, but it is proposed that some of the reactions of pyrroles with sulphuryl chloride involve radicals (14).



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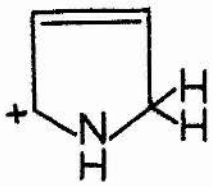

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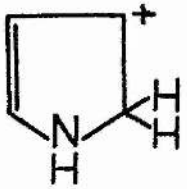

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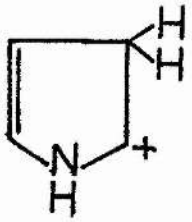

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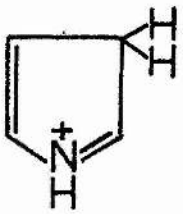

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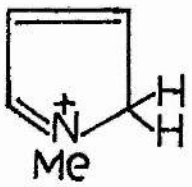
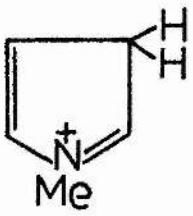


α-structure

VI



β-structure

VII

Phenyl radicals react with 1-ethoxycarbonylpyrrole to give 1-ethoxycarbonyl-2-phenylpyrrole (15).

Basicity of pyrroles. —

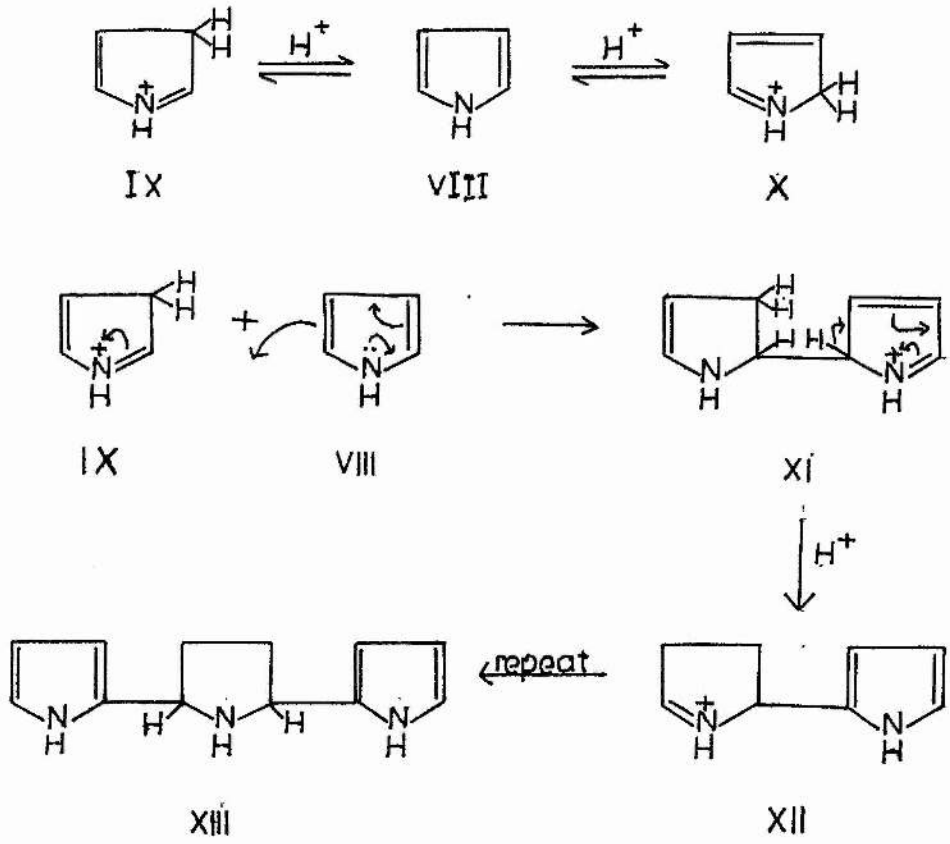
As acid-catalysed electrophilic substitution in pyrroles is the main theme of this thesis a short resumee of the effect of acid on pyrroles will be given. Pyrrole, like indole, shows unusual behaviour with respect to protonation in that it undergoes equilibrium protonation on carbon rather than on nitrogen; it has been proposed that no more than 1% of the conjugate acid of pyrrole can be nitrogen-protonated (16). Recent work indicates that pyrrole is less basic than originally suggested (17), having a pK_a value of -3.8 (18). As in electrophilic substitution, the preferred position of protonation is the α-position. This can be rationalised in terms of the resonance opportunities open to the protonated species. In the case of α-protonation, three contributing structures can be written (I, II, III). For β-protonation only two (IV, V) can be set up. Similar structures to these can be used to explain the preference of α- as opposed to β-substitution in electrophilic attack since the presumed intermediates in electrophilic substitution resemble the above species.

The effect of methyl substituents in the pyrrole ring is readily predictable. An N-methyl group increases the basicity of both α- and β-positions, the β-position being more strongly enhanced

due to the greater stabilisation of the double bonds by the methyl group in the β-structure (VI, VII). These structures also help explain the observation that N-methylpyrrole is less selective than pyrrole, with respect to the α- and β-positions, in electrophilic substitution reactions (30).

A methyl group in an α-position enhances the basicity of both the opposite α-position and the adjacent β-position whereas a β-methyl group enhances the basicity of the adjacent α-position. The basicity of the carbon atom to which the methyl group is attached is always reduced. It is therefore predictable that 2,5-dimethylpyrrole should show a relatively greater degree of β-protonation than pyrrole itself. This has been shown to be so (ca. 30% β-protonation) by observing the n.m.r. spectrum of 2,5-dimethylpyrrole in sulphuric acid (18).

It should be noted, however, that in electrophilic substitution it is very unlikely that these pyrrole cations play any active role in the reaction. Not only are they less reactive towards electrophilic attack than the neutral species but it is also very difficult to formulate any plausible reaction mechanism involving these species. Treibs (9, 19, 20) has proposed a distinctive mechanism for electrophilic substitution incorporating these cations but Smith (21) has argued convincingly against this mechanism and no support for it comes from the present study. The effect of pyrrole protonation in acid solution, however, must be taken into account in any detailed mechanistic study of electrophilic substitution as

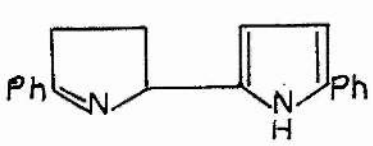


IX VIII X

IX VIII XI

XIII XII

SCHEME 1



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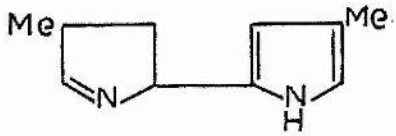

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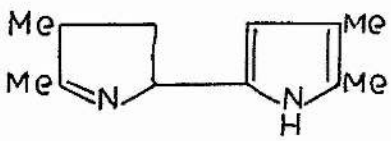


XVI

is evident from the results incorporated in this thesis. Although essentially no N-protonation occurs in solution, this may not be the case in crystalline salts (22).

Polymerisation of pyrroles.-

Polymerisation of pyrroles in acid solution is a widely reported occurrence and can complicate their study under such conditions. The situation is rather complicated as can be seen from their behaviour with ethereal hydrogen chloride. Pyrrole forms a trimer (XIII), 2-phenyl- (XIV), 3-methyl- (XV) and some 2-alkyl- and 2,3-dialkylpyrroles (XVI) form dimers (24,25,26), while some, including 2,4-dimethyl, 2,5-dimethyl and 2,3,4-trimethylpyrrole (22,25,27) give simple hydrochloride salts. Pyrroles with both α-positions substituted do not polymerise (29). The accepted reaction mechanism (Scheme 1) (23) for the formation of pyrrole trimer is informative in that it shows the β-protonated cation of pyrrole to be the more powerful electrophile and it is this species which attacks the neutral pyrrole molecule. It is obvious from this mechanism that polymerisation can only occur where both the free base and the conjugate acid are found together in solution. In weakly acidic solution none of the conjugate acid is found and in strongly acidic solution no free base is present, therefore no polymerisation can take place.

Theoretical studies.-

Many theoretical studies have been carried out on the pyrrole ring and it is interesting to compare these theoretical results with

the experimental facts outlined above. Early electron density calculations by Longuet-Higgins and Coulson (31a) using the valency-bond and molecular orbital methods determined that the π-electron density was greater at the α-position than the β-position but later work (32, 33) indicated that the β-position has the greater net negative charge. Thus, any prediction based solely on ground state atomic charges would have to be that the β-position is the more susceptible to electrophilic attack. On the other hand, however, it is found that the α-protonated cation is the more stable of the two possible protonated species (32(e), 33). In the most recent of these studies Politzer and Weinstein (33) have found it necessary to invoke out-of-plane bending of the hydrogen atoms attached to the α-carbon and the nitrogen atoms to account for the preference of α-substitution. Under these conditions it is found that a large net negative charge is generated at the α-carbon on the side opposite that of the attached hydrogen atom, and it is to this site that any approaching electrophile is drawn. Experimentally determined force constants for the above bending modes have been determined (34) and these confirm that for most of the time the N-H and C-H bonds are bent out of the plane of the ring. These ideas are in agreement with the widely held view that electrophilic substitution reactions proceed via a tetrahedral complex, or 'Wheland' intermediate. One drawback of this work is that it did not take into account localisation energies. These are, however, discussed in Chapter 3 of the present work.

Chapter 1

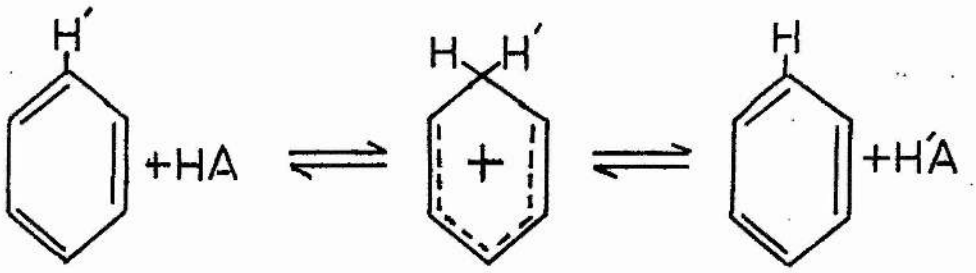
Acid-catalysed hydrogen-exchange in pyrroles

Introduction.-

Hydrogen exchange is the general term used to describe reactions in which one isotope of hydrogen is replaced by another and is the simplest form of electrophilic substitution. The exchange reactions include deuteration and tritiation in which hydrogen is replaced by deuterium and tritium, respectively, and protodedeuteration and protodetritiation in which deuterium and tritium, respectively, are replaced by hydrogen.

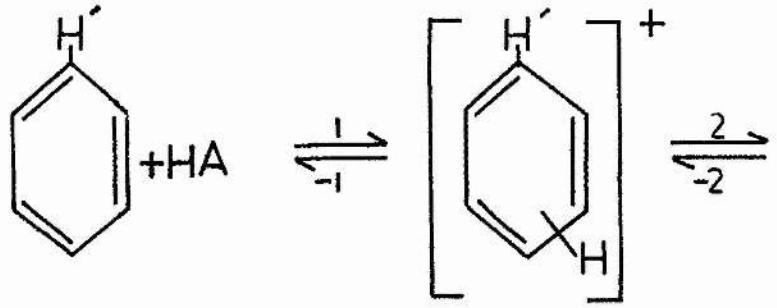
Protodedeuteration and protodetritiation have several distinct advantages over other types of reactions in studying the mechanism of electrophilic aromatic substitution. Firstly, by a suitable choice of synthetic methods, it is possible to 'label' one position of a molecule and study the reactivity at that position irrespective to its relative reactivity with respect to the other positions in the molecule. For example, it is possible to determine the rates of protodetritiation accurately for both the 3- and 4-positions in toluene using 3- and 4-tritiotoluene. However, the study of the relative reactivities of these two positions in a reaction such as halogenation is difficult due to problems in measuring the relatively small amounts of 3-isomer produced in the reaction.

Secondly, it is possible to vary the experimental conditions widely for exchange reactions to give large changes in the reactivity and therefore selectivity of the reagent (acid). This makes it possible to obtain information about the way in which the effect of a substituent, whether activating or deactivating, depends on the activity of the reagent.



XVII XVIII XIX

SCHEME 2



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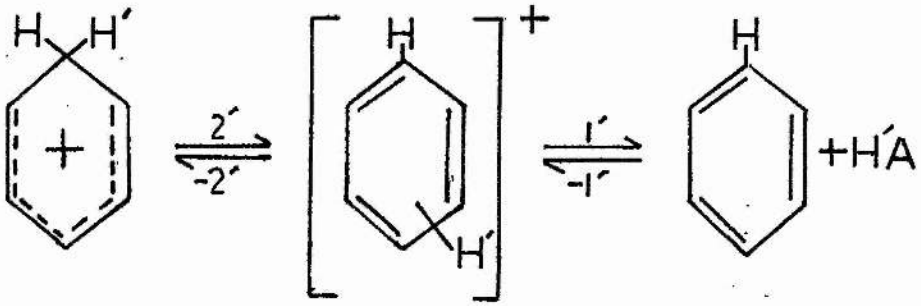


XXI XXII XIX

SCHEME 3

Finally, hydrogen exchange, to a first approximation, is free of steric effects which makes it particularly useful for studying the effects of ortho-substituents.

Two mechanisms have been proposed for hydrogen-exchange in aromatic systems. The first, designated A-S_E2 (Scheme 2), is that most commonly used to explain electrophilic aromatic substitution. It consists of a bimolecular process between the acid, HA, and the aromatic ring, resulting in the formation of a 'Wheland' intermediate, followed by loss of a proton.

The second mechanism (Scheme 3) was first proposed by Gold and Satchell (35). Steps 1 and 1' are fast and 2 and 2' are rate determining; XX and XXII are outer, or π-complexes, and XXI is an inner, or σ-complex ('Wheland' intermediate); and in XX, XXI and XXII the species A^- has lost its association with the exchanging proton. This mechanism is designated A-1.

The formation of a 'Wheland' intermediate is common to both mechanisms and good evidence for its existence has been found using n.m.r. studies which have shown the presence of aliphatic CH_2-groups (36).

Evidence to support an A-1 mechanism is found in the observation by Gold and Satchell (35) that there is a linear relationship between log k and $-H_0$ (the Hammett acidity function) for the protodedeuteration of anisole, some phenols, toluene and benzene and this, according to the Zucker-Hammett hypothesis (37), indicates that water cannot be present in the transition stage (which would be the case if HA is H_3O^+). It is claimed, therefore, that

this observation supports an A-1 mechanism in that A^- has separated from the aromatic system before the slow step. To be used as a rigorous mechanistic criterion, however, the slopes of these lines should be unity which is not the case. Other workers (37, 38, 39), studying different reactions, have also found a linear relationship between log k and $-H_o$ but with slopes differing from unity and since the Zucker-Hammett hypothesis is now open to doubt (40) it can no longer be invoked in order to reject an A-S_E2 mechanism.

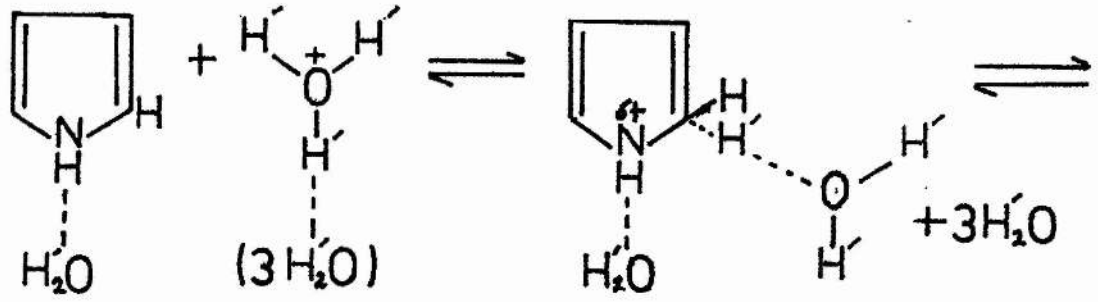
One of the main pieces of evidence in support of an A-S_E2 mechanism for hydrogen exchange is the detection of general acid catalysis, first reported by Kresge and Chiang (41) for the protodetritiation of 1, 3, 5-trimethoxybenzene and extended by other workers to azulenes (42), indoles (43) and thiophens (39). The reaction is catalysed by various A^- species present, which is inconsistent with an A-1 mechanism and is in agreement with an A-S_E2 mechanism.

Although the above evidence is strongly in support of an A-S_E2 mechanism for hydrogen exchange, Melander (44) has suggested that there may be a change in mechanism from A-S_E2 to A-1 according to the strength of the acid medium and the reactivity of the aromatic position and Gold (45) has come to a similar conclusion.

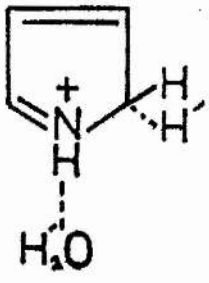
Hydrogen exchange occurs at the three possible positions in the pyrrole ring; at the nitrogen and at both the α- and β-positions (18, 46, 47, 48, 49). Early work by Koizumi and Titani (46) showed that hydrogen exchange at the nitrogen atom was much faster than

exchange at either carbon atoms. However, Muir and Whiting (49) have determined that exchange at the heteroatom to be only 10-20 times faster at than at the carbon atoms. The relative exchange rate at the two different carbon atoms is more complex. Schwetlick et al (47) found that the relative exchange rate for the α- and β-positions (α/β) to be 2.5 in a methanol:water:sulphuric acid mixture, Muir and Whiting (49) found the ratio to be about 1 in an acetonitrile:water:perchloric acid mixture and Bean (48) determined it to be 1.6 in a dioxane:water:acetic acid mixture. However, Bean (48) also found that in a more acidic medium containing trifluoroacetic acid hydrogen exchange at the β-position was faster than at the α-position. A similar result was also observed by Whipple, Chiang and Hinman (18) with N-methylpyrrole in concentrated sulphuric acid, whereas in a weakly acidic solution they noted that exchange is faster at the α-position. Bean (48) has therefore proposed that with increased acidity the transition state occurs early and thus resembles the neutral pyrrole molecule, where it has been calculated (see general introduction) that there is a greater electron density at the β-position, rather than its conjugate acid. In weaker acid the transition state would be later and therefore resemble the conjugate acid, where it is predicted (see general introduction) the conjugate acid formed by α-protonation would be more stable than that formed by β-protonation.

The work by Muir and Whiting (49) in which the activity of the water is varied, shows that water molecules are associated with

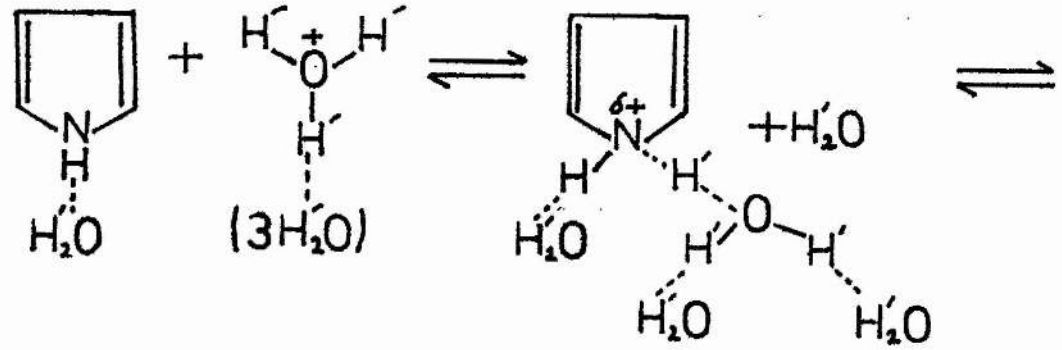


XXIII XXIV



C-H exchange

XXV



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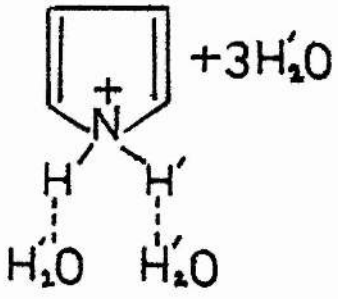


N-H exchange

XXVII

SCHEME 4

the transition stage and therefore indicates an A-S_E2 mechanism as opposed to an A-1 mechanism for hydrogen exchange in the pyrrole ring. Their mechanisms for C-H exchange and N-H exchange are given in Scheme 4.

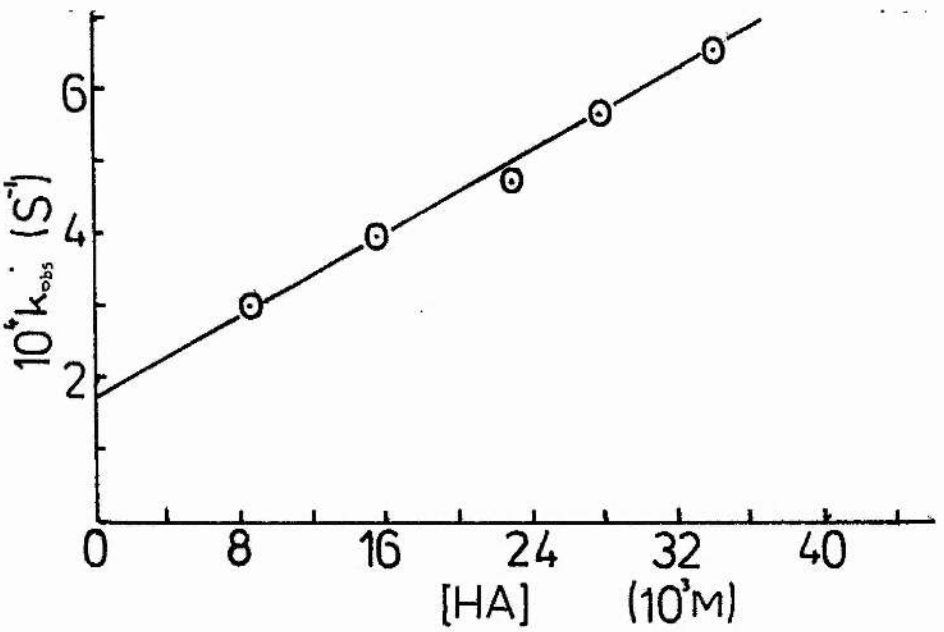


FIGURE 1 Variation of k_{obs} with buffer concentration at constant pH for protodetritiation of 1,3,4-trimethyl-2,5-ditritiopyrrole.

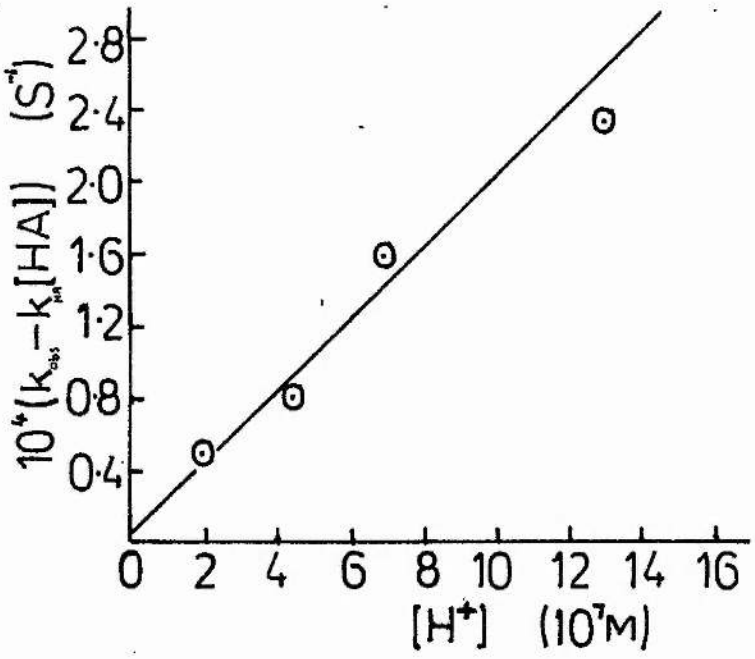


FIGURE 2 Variation of $(k_{obs}-k_{aa}[HA])$ with hyrogen ion concentration for protodetritiation of 1,3,4-trimethyl-2,5-ditritiopyrrole.

Results and discussion.-

The present study into hydrogen exchange in pyrroles represents an attempt to detect general acid catalysis for this reaction and thereby proving that it proceeds by an $A\text{-}S_E2$ mechanism. The method employed was protodetritiation using liquid scintillation counting. The pyrroles used were 1,2,5-trimethyl- and 1,3,4-trimethylpyrrole. Since both these pyrroles contain only one type of ring proton the synthesis of the tritiated species were simple and the resulting kinetics easily interpreted. The buffer solution used was a potassium dihydrogen phosphate:sodium hydroxide mixture. A brief summary of the theory of acid-base catalysis is given in Appendix 1, the equation for general acid catalysis being

$$k_{obs} = k_o + k_{H^+}[H^+] + k_{HA}[HA],$$

where k_{obs} is the measured rate constant, k_o is the uncatalysed rate constant and the terms k_{H^+} and k_{HA} are called catalytic coefficients.

The results of protodetritiation of 1,3,4-trimethyl-2,5-ditritiopyrrole are given in Tables 1 and 2 and shown in Figures 1 and 2.

From Figure 1 the value of k_{HA} was calculated to be $1.43 \times 10^{-2}\ s^{-1}M^{-1}$. This value was used for the calculations in Table 2.

Table 1

Variation in the rate of protodetritiation of 1,3,4-trimethyl-2,5-ditritiopyrrole with buffer concentration at constant pH and at 25°C

[HA] (M)	pH	$10^4 k_{obs}$ (s^{-1})
0.0337	6.151	6.55
0.0278	6.154	5.58
0.0225	6.156	4.84
0.0156	6.158	3.99
0.0088	6.160	3.05

Table 2

Variation in the rate of protodetritiation of 1,3,4-trimethyl-2,5-ditritiopyrrole with pH at 25°C

[HA] M	$10^4 k_{HA}[HA]$ (s^{-1})	$10^4 k_{obs}$ (s^{-1})	$10^4(k_{obs} - k_{HA}[HA])$ (s^{-1})	pH	$10^7 [H^+]$ (M)
0.0262	3.73	6.09	2.35	5.89	12.8
0.0225	3.21	4.84	1.60	6.15	6.98
0.0188	2.68	3.48	0.80	6.33	4.68
0.0106	1.51	1.98	0.47	6.69	2.04

From Figure 2 the value of k_{H^+} was calculated to be 1.94×10^2 $s^{-1}M^{-1}$ and k_o was found to be so small as to be essentially zero.

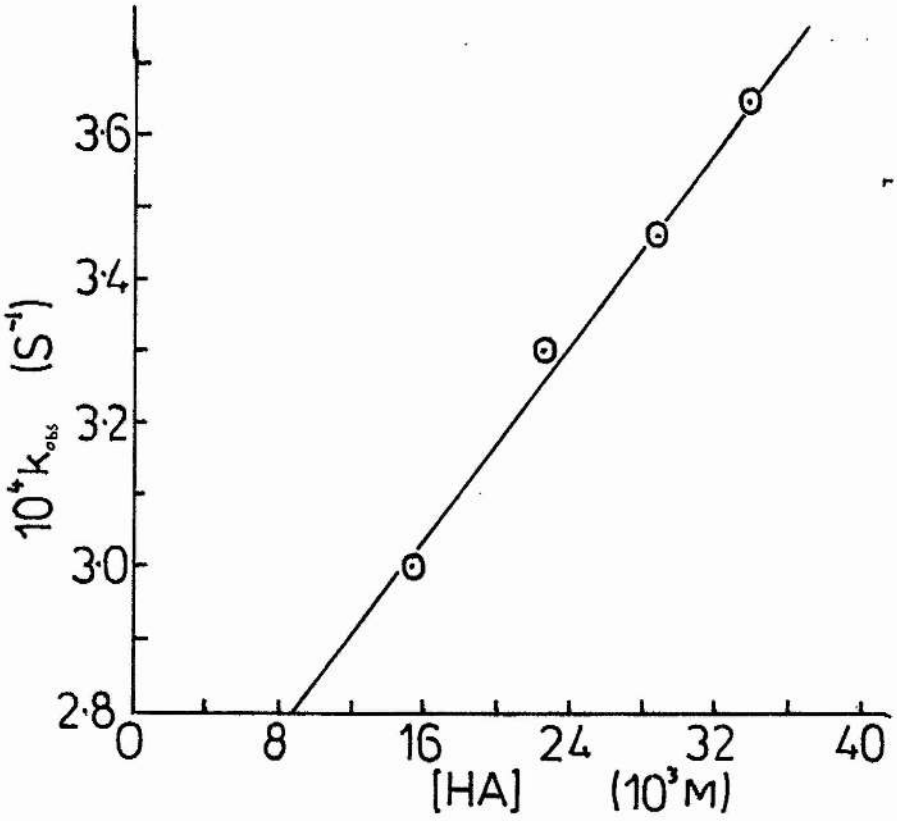


FIGURE 3 Variation of k_{obs} with buffer concentration at constant pH for protodetritiation of 1,2,5-trimethyl- 3,4-ditritiopyrrole.

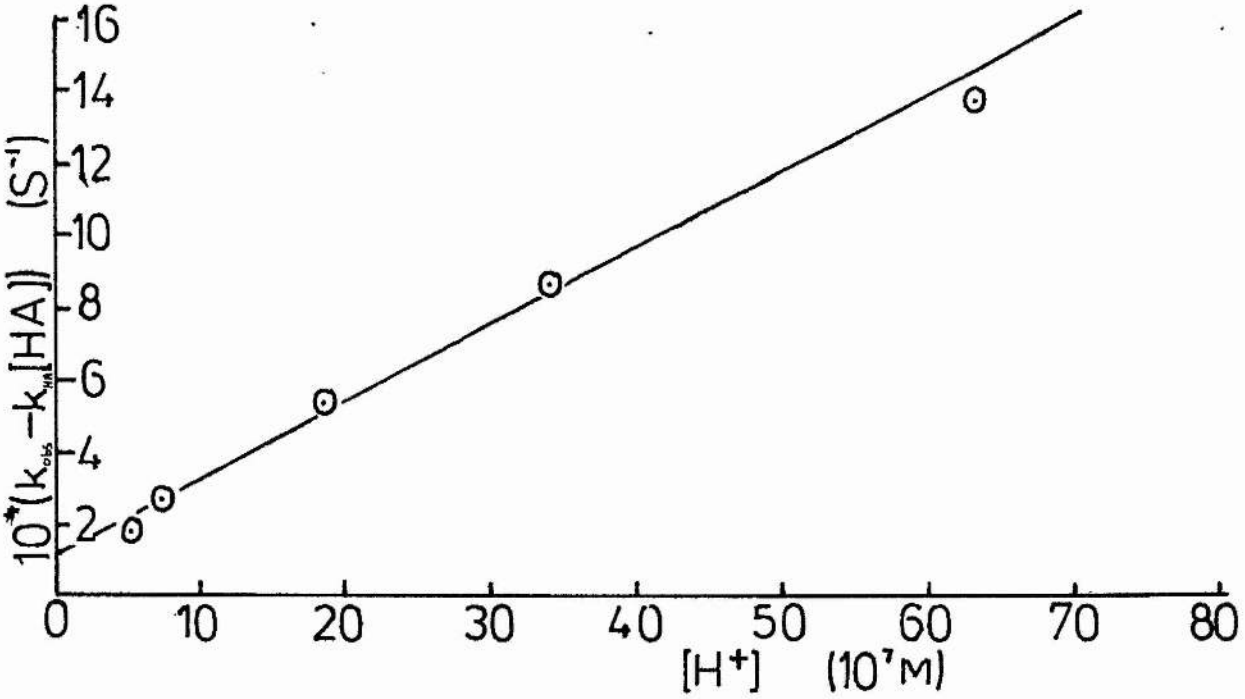


FIGURE 4 Variation of $(k_{obs} - k_{HA}[HA])$ with hydrogen ion concentration for protodetritiation of 1,2,5-trimethyl- 3,4-ditritiopyrrole

The results of protodetritiation of 1,2,5-trimethyl-3,4-ditritiopyrrole are given in Tables 3 and 4 and shown in Figures 3 and 4.

Table 3

Variation in the rate of protodetritiation of 1,2,5-trimethyl-3,4-ditritiopyrrole with buffer concentration at constant pH and at 25°C

[HA] (M)	pH	$10^4 k_{obs}$ (s^{-1})
0.0337	6.155	3.63
0.0289	6.163	3.46
0.0225	6.163	3.27
0.0156	6.147	3.01

From Figure 3 the value of k_{HA} was calculated to be 3.33×10^{-3} $s^{-1}M^{-1}$. This value was used for the calculations in Table 4.

Table 4

Variation in the rate of protodetritiation of 1,2,5,-trimethyl-3,4-ditritiopyrrole with pH at 25°C

[HA] (M)	$10^5 k_{HA}[HA]$ (s^{-1})	$10^4 k_{obs}$ (s^{-1})	$10^4(k_{obs}-k_{HA}[HA])$ (s^{-1})	pH	$10^7 [H^+]$ (M)
0.0330	11.0	14.8	13.7	5.198	63.4
0.0309	10.3	9.42	8.39	5.467	34.1
0.0288	9.63	6.31	5.35	5.730	18.6
0.0225	7.50	3.27	2.52	6.163	6.87
0.0188	6.27	2.54	1.91	6.285	5.19

From Figure 4 the value of k_{H^+} was calculated to be $2.08 \times 10^2 s^{-1}M^{-1}$ and again k_o was found to be essentially zero.

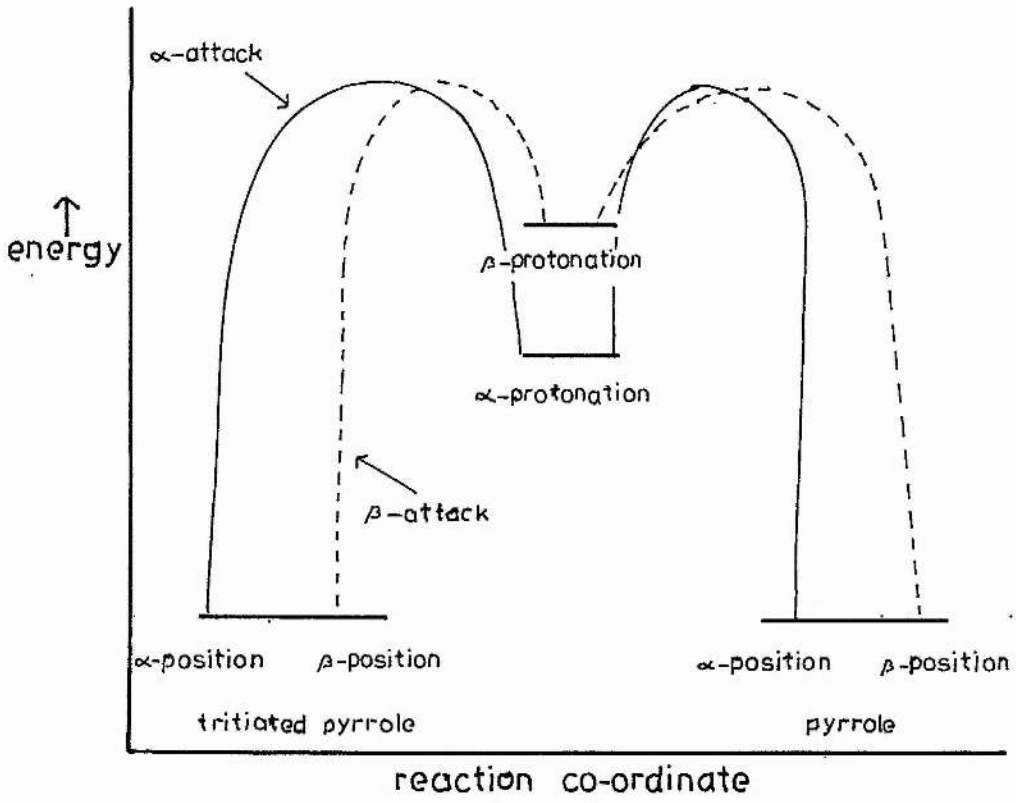


FIGURE 5

Energy profile for hydrogen-exchange in pyrrole

These results show that hydrogen-exchange in pyrroles is subject to general acid catalysis and therefore confirm an A-S_E2 mechanism for this reaction.

It is evident from the previous studies into hydrogen exchange in pyrroles, and also by the present work, that the exchange rates at the α- and β-positions are similar in magnitude; the relative rate, in fact, can be reversed by changing the experimental conditions. This seems to conflict with the known preference for α-substituted products from electrophilic substitution reactions. However, it should be noted that the equilibrium distribution of the initial pyrrole reactant and the α- and β-substituted products depends mainly on the relative stabilities of these species and is independent of the kinetics of the reaction. Thus, although hydrogen exchange takes place more rapidly at the heteroatom of pyrrole, in any equilibrium mixture of pyrrole in acid the N-protonated form will only account for ca. 1% of the total protonated pyrrole, the remainder being C-protonated. This is because the relative stabilities of the three protonated species are α-C-protonation $>\beta$-C-protonation $>$N-protonation.

The energy profile for hydrogen-exchange in pyrrole can be drawn as in Figure 5. In this reaction the starting material and product have essentially the same ground state energy. The fact that the rates of hydrogen-exchange at the two positions are similar means that the activating energy for exchange at the two positions must also be essentially the same. It should be noted that in the

protodetritiation reaction carried out there is virtually no chance of any back-reaction being set up. Once the tritium has been replaced in the reactant it becomes so diluted by protium ions that any subsequent 'protonation' of the pyrrole must be by a protium ion. Therefore, at any given time, the ratio of the products to reactant is dependent on the kinetics of the reaction rather than the thermodynamic properties of the species. This is in contrast to the situation found when a reaction is carried out under synthetic conditions.

In the general introduction it was noted that the β-position of pyrrole has the greater net negative charge associated with it and, therefore, on the basis of this, should be the more likely position to undergo electrophilic substitution. Politzer and Weinstein (33) have shown, however, that by bending the hydrogen atoms attached to the α-carbon and nitrogen atoms of the ring an extensive region of negative charge is generated in the vicinity of the heteroatom and the α-carbon. Although the region surrounding the β-carbon is still the more electronegative, Politzer and Weinstein have proposed that any approaching electrophile would be preferentially attracted to the extensive negatively charged region surrounding the α-carbon. Thus the preference for α-substitution is explained. This conclusion seems premature. It appears more likely that any such ring deformation would only help offset the greater electronegativity of the β-position and therefore the rate of attack at both positions may be similar. In the case of N-methylpyrrole it is suggested that, since the methyl

group is more difficult to move out of the plane of the ring, this molecule would be less selective than pyrrole as regards the position of electrophilic attack. This is, in fact, found experimentally.

A similar argument can be extended to include the pyrroles used in this study. For 1, 2, 5-trimethylpyrrole the bending of the methyl groups out of the ring plane is even more difficult and therefore the ratio of the rates of α- to β-substitution would be smaller than the same ratio for pyrrole. This is, of course, a hypothetical argument in that α-attack on 1, 2, 5-trimethylpyrrole does not lead to any newly substituted product. In the case of 1, 3, 4-trimethylpyrrole the N-methyl group should have the same effect as that in N-methylpyrrole. The β-methyl groups, however, would certainly diminish the chances of the β-position deriving any additional increase in electronegativity by out-of-plane bending of these groups. Thus, relative to unsubstituted pyrrole, it would be predicted that the ratio of the rates of α- to β-substitution would be greater in this compound. It is predicted, therefore, that the relative rate of α-hydrogen exchange in 1, 3, 4-trimethylpyrrole to β-hydrogen exchange in 1, 2, 5-trimethylpyrrole would be similar to the relative rate of α- to β-hydrogen exchange in pyrrole itself. This would suggest that the rates of exchange at the two positions studied in this present work would be similar in magnitude, which is found experimentally. The above argument, of course, assumes that the inductive effect of an α-methyl group on the adjacent β-position is the same as that of a β-methyl group on the corresponding α-position.

The pK_a values of the pyrroles studied must now be considered since these may be thought to play an important role in the rate of hydrogen exchange. The equilibrium constant K for any reaction can be expressed as the ratio of the rate of the forward reaction (k_1) to that of the backward reaction (k_{-1}). Since in hydrogen exchange the value of k_1 (protonation) would be rate determining the value of the pK_a may reflect this rate. Unfortunately, however, the values of k_{-1} are unavailable and therefore any further consideration of this topic would be pointless. The experimental evidence, however, indicates that the rate of hydrogen exchange at any position in the pyrrole ring is independent of the pK_a of that position. Thus for pyrrole, where the pK_a values are ca. -10 for N-protonation, -5.9 for β-C-protonation and -3.8 for α-C-protonation, the previous work on the rates of hydrogen exchange does not reflect this large range in basicity. Similarly, the present work on methyl-substituted pyrroles (where for 1,2,5-trimethylpyrrole the pK_a values are -0.21 for α-C-protonation and -0.10 for β-C-protonation and for 1,3,4-trimethylpyrrole the values are 1.4 for α-C-protonation and -4.6 for β-C-protonation) indicates that rates of hydrogen exchange are independent of the pK_a values.

A similar argument to that for hydrogen exchange can be extended to the reaction of pyrroles with Ehrlich's aldehyde (see Chapter 2) in acid solution. For 2,5-dimethyl- and 1,2,5-trimethyl-pyrrole the reaction must take place at the β-position. These reactions are very fast, however, and were followed using the same stopped-flow time scale as the other pyrroles (where α-substitution predominates) and indeed the rate (see Table 6 in

Chapter 2) of reaction for 1,2,5-trimethylpyrrole is only slightly less than that for 1,2-dimethylpyrrole.

It should also be noted that for both these pyrroles there is a substantial back-reaction. Both these findings are in agreement with what has been written above for hydrogen exchange reactions in pyrroles.

It has been established that electrophilic substitution (including hydrogen exchange) can take place at alkyl groups attached to aromatic systems (50, 51, 52, 53, 54, 55, 56). This will be discussed more fully in Chapter 3. It seemed pertinent, therefore, to determine whether or not hydrogen exchange occurs at the methyl groups attached to polymethyl pyrroles. Should it occur, this could explain why 2,3,4,5-tetramethylpyrrole reacts with electrophiles such as 4-dimethylaminobenzaldehyde (57) (see Chapter 2) and diazonium salts (57). The pyrroles used in this study were 2,3,4,5-tetramethylpyrrole, 2,5-dimethylpyrrole and 2-methylpyrrole and the experimental method employed was to dissolve the pyrroles in deuteriotrifluoroacetic acid and to study any changes in their ^{1}H n.m.r. spectra as a function of time. An internal standard of methylene chloride was incorporated in the n.m.r. samples and the integral values of the methyl group peaks were compared with this standard. Under these conditions any hydrogen atoms attached directly to the ring had exchanged before the first spectra could be run.

For tetramethylpyrrole the spectra obtained were exactly the same as the spectrum in sulphuric acid obtained by Chiang and Whipple (58), indicating that 2-protonation had occurred. Over a period of 4 hours it was found that the integral values of the methyl group peaks had not varied indicating that no exchange had occurred in these groups. Again the series of spectra obtained for 2, 5-dimethylpyrrole were similar to that obtained by Chiang and Whipple (58), indicating in this case that both 2- and 3-protonation had occurred. No hydrogen exchange had occurred at the methyl groups after a period of 4 hours. In the case of 2-methylpyrrole, when the trifluoroacetic acid was added to the pyrrole a rather violent reaction occurred and the resulting spectrum showed a large number of ill-defined peaks which could not be accounted for. The experiment was repeated but the spectrum obtained was no more easily explained. This is in contrast to the result obtained by Chiang and Whipple (58) where they obtained a spectrum showing protonation to have taken place at the 5-position. Polymerisation of the pyrrole may be the cause of the result obtained in trifluoroacetic acid but it is difficult to see why a similar result would not be found in sulphuric acid.

In an additional experiment on 2, 3, 4, 5-tetramethylpyrrole the ^{13}C n.m.r. spectrum in chloroform was taken. It is known (59) that the ^{13}C n.m.r. spectra of substituted pyrroles show that the pyrrole ring ^{13}C chemical shifts follow additivity, depending on the nature of the substituents and interactions between them. It

was thought possible that the four methyl groups in tetramethylpyrrole may set up such steric interactions that the pyrrole ring would be distorted from its planar configuration and therefore the ring ^{13}C chemical shifts would not follow the pattern outlined above. If such a distortion did occur it may help explain the reactivity of this compound. The theoretical ring ^{13}C chemical shifts calculated using the method above (59) are as follows.

For the α-carbon - effect of methyl groups on chemical shifts

117.3 (the chemical shift of the α-carbon in unsubstituted pyrrole)

+ 10.8 (2-methyl substituent)

+(-2.1) (5-methyl substituent)

+(-2.4) (3-methyl substituent)

+(-0.9) (4-methyl substituent)

= 122.7 p.p.m.

effect of methyl group interactions

122.7 + (-0.7) (2-methyl:3-methyl group interaction)

+ 0.2 (4-methyl:5-methyl group interaction)

+(-0.1) (3-methyl:4-methyl group interaction)

= 122.1 p.p.m.

For the β-carbon - effect of methyl groups on chemical shifts

107.6 (the chemical shift of the β-carbon in unsubstituted pyrrole)

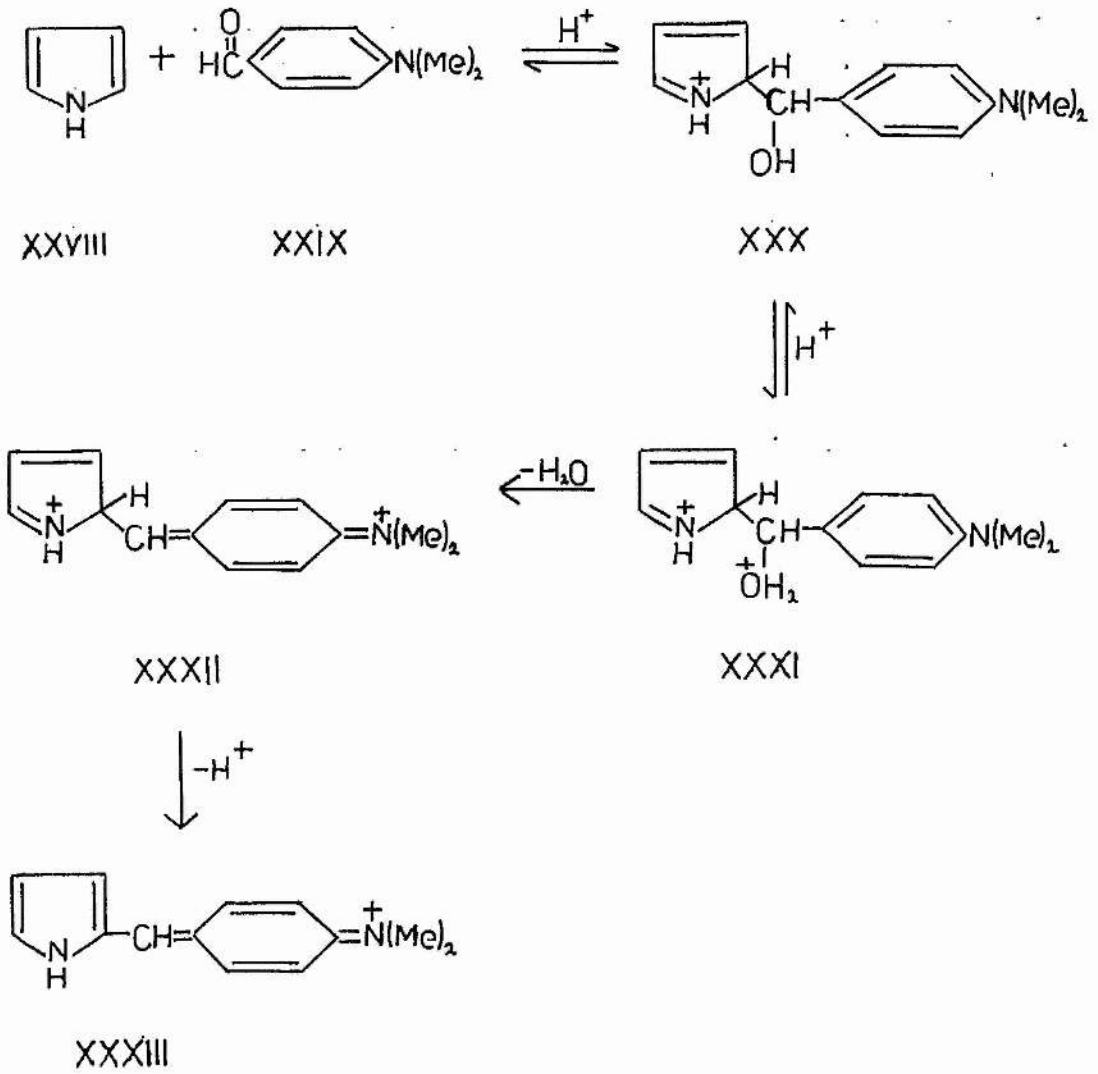
+(-1.2) (2-methyl substituent)

+ 1.1 (5-methyl substituent)

+12.7 (3-methyl substituent)

+ 1.1 (4-methyl substituent)

= 121.3 p.p.m.



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SCHEME 5

effect of methyl group interactions

121.3 + (-3.8) (2-methyl:3-methyl group interaction)

+ (-0.1) (4-methyl:5-methyl group interaction)

+ (-2.8) (3-methyl:4-methyl group interaction)

= 114.6 p.p.m.

These theoretically calculated values of 122.1 p.p.m. and 114.6 p.p.m. compare favourably with the corresponding experimental values of 120.7 p.p.m. and 113.8 p.p.m. relative to a TMS reference.

The ^{1}H n.m.r. results would therefore suggest that electrophilic substitution does not occur at methyl groups attached to the pyrrole ring and the ^{13}C n.m.r. experiment indicates that there are no unusual structural features associated with tetramethylpyrrole to account for it undergoing electrophilic substitution reactions. Treibs et al (60) have proposed that the reaction between tetramethylpyrrole and 4-dimethylaminobenzaldehyde proceeds with loss of a methyl group as methanol (in an analogous process to the loss of water at an unsubstituted position (Scheme 5)). The last part of this scheme, however, involves the loss of a proton and it is difficult to see how a methyl group can be lost in a similar process. To confirm a mechanism in which a methyl group is lost, the products from the reactions between 2,3,4,5-tetramethylpyrrole and 2,3,4-trimethylpyrrole with 4-dimethylaminobenzaldehyde would have to be prepared and compared. This represents a challenging piece of synthetic chemistry as the products from these reactions prove extremely difficult to purify and characterise (see Chapter 2).

Experimental.-

Materials.-

2,5-Dimethylpyrrole was purchased and 1,2,5-trimethylpyrrole was prepared from this using the method for N-methylation described by Hinman and Theodoropulos (61). 1,3,4-Trimethylpyrrole (61) and 2,3,4,5-tetramethylpyrrole (62) were prepared using literature methods. The buffer solution used was that described by Robinson and Stokes (53) using ARpotassium dihydrogen phosphate and AR sodium hydroxide. Deuteriotrifluoroacetic acid was obtained by reaction of trifluoroacetic anhydride with deuterium oxide.

2,5-Dimethyl-, 1,2,5-trimethyl- and 1,3,4-trimethylpyrrole were vacuum distilled and 2,3,4,5-tetramethylpyrrole was recyrstallised repeatedlyfrom hexane to constant melting point prior to use.

Tritiodeprotonation of pyrroles.-

The appropriate pyrrole (0.5 g) was added to a mixture of 49% sulphuric acid (1 ml) and tritiated water (1 ml; 50 mC/ml) and stirred for 1 hour. The resultant solution was added to an excess of 0.5 M sodium hydroxide and extracted with ether (50 ml). The ether extracts were washed with distilled water (2 x 25 ml), dried ($MgSO_4$) and evaporated to give the tritiated pyrrole.

The tritiated pyrroles were vacuum distilled before being used for the kinetic studies.

Kinetics.-

An aqueous solution of the required pyrrole was prepared by adding a drop of the tritiated pyrrole to 100 ml of distilled water, stirring for $\frac{1}{2}$ hour and filtering the solution through phase separating paper to remove any undissolved pyrrole. 10 ml of the aqueous solution was added to a 50 ml volumetric flask and to this was added the necessary buffer solution to give the required buffer and/or acid concentration. The ionic strength of this solution was kept constant at 0.53 M by adding calculated quantities of AR potassium chloride. The flask was stoppered and thermostatted in a water bath. A 1 ml aliquot of the reaction mixture was taken and added to an excess of 0.2 M sodium hydroxide. This was extracted with 10 ml of a toluene based scintillation solution, the organic layer separated, washed with distilled water (25 ml) and dried over magnesium sulphate. A 5 ml aliquot was taken and counted using a Beckman LS100 Counter. Similarly, further 1 ml aliquots were taken from the reaction mixture at convenient time intervals, and prepared for counting as before. The error in the counting system was pre-set at 2%.

The pHs of the reaction mixtures were measured using a Beckman Research pH Meter and glass and calomel electrodes.

As a check to ensure that no decomposition of the pyrrole solution had taken place during each kinetic run, a 1 ml aliquot of each solution was reacted with 1 ml of 0.25 M 4-dimethylamino-benzaldehyde solution (see Chapter 2) before and after each kinetic

run. The optical densities of the resulting coloured solutions were the same indicating that no decomposition had taken place.

The rate constants were calculated by the method of Swinbourne (65) and Kezdy (64) (see Appendix 2).

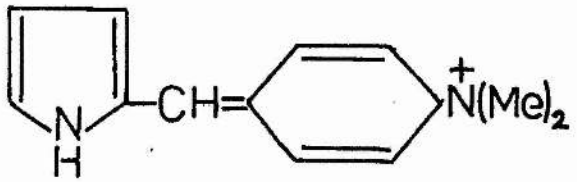
N.m.r. studies.-

A mixture of the pyrrole and methylene chloride as internal standard (roughly 50:50) was prepared and its ^{1}H n.mr. in $CDCl_3$ was taken with an internal reference of TMS. The integral ratio of the methylene chloride resonance with respect to that of the methyl group(s) of the pyrrole was measured. A series of spectra of the same pyrrole/methylene chloride mixture were taken in deuteriotrifluoroacetic acid as a function of time and any decrease in the integral value of the methyl group(s) attached to the pyrrole was measured relative to the methylene chloride integral.

The ^{13}C n.m.r. spectrum of 2,3,4,5-tetramethylpyrrole was taken with chloroform as solvent and an internal reference of TMS.

Chapter 2

Reaction of pyrroles with 4-dimethylaminobenzaldehyde (Ehrlich's Reagent) in acid solution.



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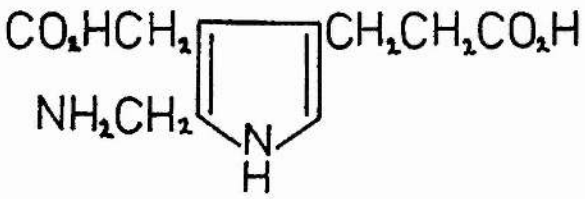

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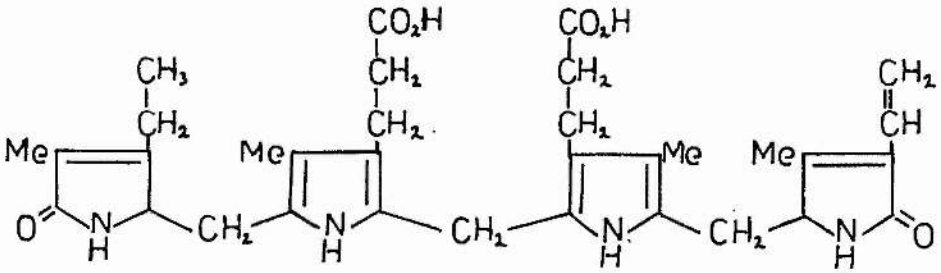

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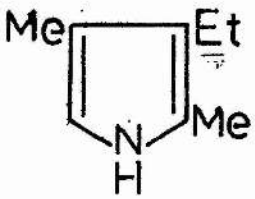


XXXVI

Introduction.-

Although a sample of pure pyrrole was not obtained until 1857, in 1834 Runge noticed that when a pine splinter was moistened with mineral acid and exposed to the vapours from the distillation of coal tar, bones and similar materials a bright red colour appeared. He, subsequently, called the substance giving this colour reaction, pyrrole (Gr. pyrros, red). The red colour produced is due to reaction between the pyrrole and aldehydes present in the wood. In 1901 Ehrlich (66) modified this reaction by replacing the pine splinters by 4-dimethylamino-benzaldehyde (DMAB) and this colour reaction still bears his name.

The proposed structure of the product formed in this reaction is shown (XXXIII) and evidence to support this is discussed later.

It is not true, as commonly stated, that all pyrroles with an unsubstituted α-position will react and the performance in this colour test parallels roughly the ability to couple with diazonium salts (9, 67, 68). Thus, of the four compounds ethyl pyrrole-1-carboxylate, ethyl 4-nitropyrrole-2-carboxylate, diethyl 3-methyl-pyrrole-2,4- and diethyl 2-methylpyrrole-3,5-dicarboxylate, none of which undergo coupling with diazonium salts, the first three do not give the Ehrlich reaction and the last responds only on warming.

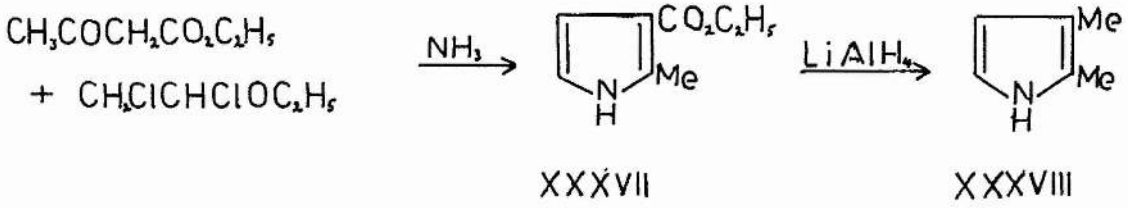
The Ehrlich reaction is not specific to pyrroles and many compounds containing the pyrrole moiety give a positive result.

This makes the test a very useful tool in the detection of many clinically important compounds. Thus, it is used in the detection of various porphyrin precursors including porphobilinogen (69) (XXXIV) and urobilinogen (70) (XXXV), the detection of urea in biological fluids (71, 72) and of various alkaloids (73, 74). The reaction became of further significance when it was found that the urine of individuals suffering from psychotic illnesses contains cryptopyrrole (XXXVI), which is known as the 'mauve factor' by virtue of its reaction with DMAB (75). More recently reaction with DMAB has been used in the detection of various hallucinogens (76). However, the reagent is not a particularly good one for these purposes as the colours developed fade fairly rapidly (77) and the test cannot be used quantitatively.

The aims of the present work were, firstly, to study the kinetics of the Ehrlich reaction with various methyl pyrroles and, secondly, to develop an improved colourimetric test for pyrroles. The first part of this work is outlined in this chapter and the latter is discussed in Chapter 4.

In the course of this study it was found necessary to prepare a fairly extensive range of methylpyrroles and the general synthetic method is outlined below. This was based on the method described by Hinman and Theodoropulos (61) in which pyrroles containing carboxyl and carbethoxy groups are reduced in good yields to the corresponding methylpyrroles using a large excess of lithium aluminium hydride as reducing agent. Although alkyl pyrroles had been prepared previously by Wolff-Kishner reductions (78)

2,3-dimethylpyrrole



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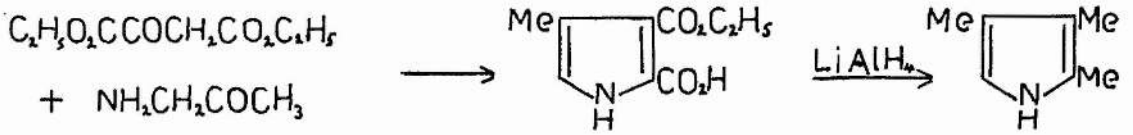
2,3,4-trimethylpyrrole



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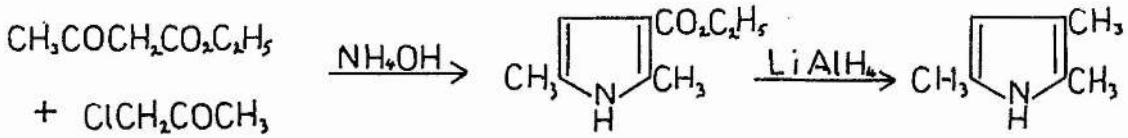
2,3,5-trimethylpyrrole



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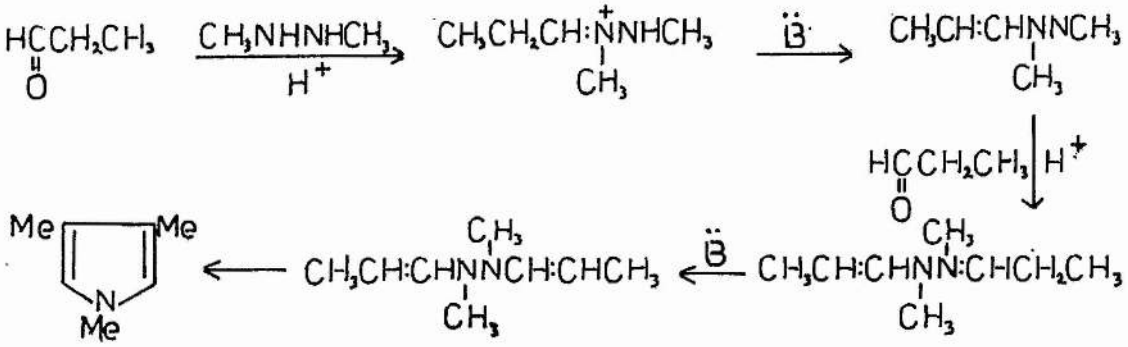
SCHEME 6



XLIII

SCHEME 7

of the corresponding carbonyl containing pyrrole, the lithium aluminium hydride method has the advantage that it offers a much greater freedom in the choice of starting materials.

The initial pyrrole compounds were prepared using Knorr or Hantzch (79) type syntheses involving, in the former case, the condensation of a β-keto-ester or a β-diketone with an α-amino ketone and, in the later, the combination of a βketo-ester with an α-haloketone in the presence of ammonia or a primary amine. A few examples of pyrrole syntheses are shown in Scheme 6 to illustrate these synthetic points.

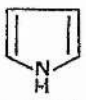
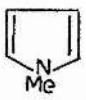
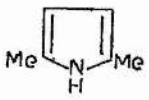
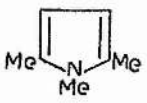
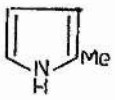
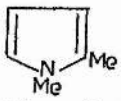
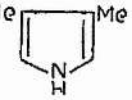
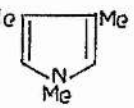
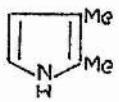
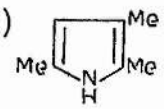
It should be noted, however, that the preparation of N-methylpyrroles from C-acyl-N-methylpyrroles using this method fails regardless of whether the acyl group is at the 2- or the 3-position. This is because reduction stops at the hydroxymethyl stage. These N-methylpyrroles were synthesised, however, from the corresponding C-methylpyrroles by treating the latter, firstly, with potassium to form the potassium salt and then with methyl iodide. A one step synthesis of N-methylpyrroles is described by Chapelle et al (80) in which 1,2-dimethylhydrazine is condensed with various aldehydes and ketones in the presence of 4-toluene-sulphonic acid. This method was used to prepare a sample of 1,3,4-trimethylpyrrole using 1,2-dimethylhydrazine and propanal (Scheme 7).

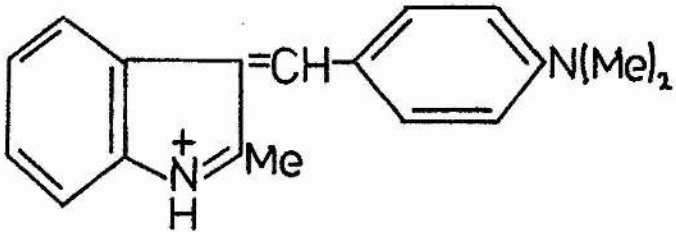
Results and discussion.-

The reaction between DMAB and dilute aqueous solutions of pyrroles in the presence of acid leads to the formation of a brilliant red or purple solution. The absorption spectra of these coloured solutions were taken and the results are given in Table 5.

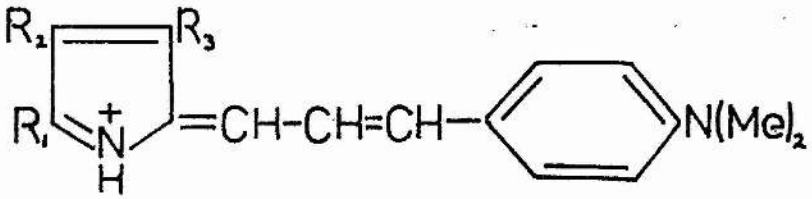
Table 5

The maximum absorptions (λ_{max}) of the coloured species obtained in the reaction of DMAB with pyrroles in acid solution.

Pyrrole	λ_{max}(nm)	Pyrrole	λ_{max} (nm)
(a)  parent	560	(b)  1-methyl	558
(c)  2, 5-dimethyl	520	(d)  1, 2, 5-trimethyl	528
(e)  2-methyl	525	(f)  1, 2-dimethyl	535
(g)  3, 4-dimethyl	515	(h)  1, 3, 4-trimethyl	520
(i)  2, 3-dimethyl	552	(j)  2, 3, 5-trimethyl	524

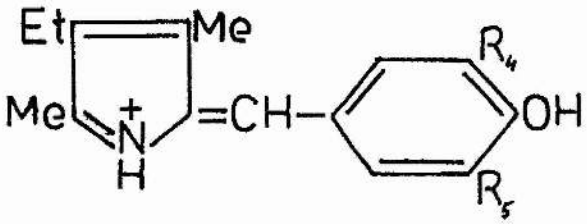


XLIV



$R_1R_2R_3$=alkyl groups

XLV



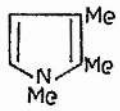
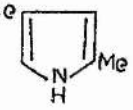
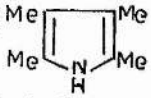
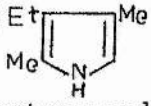
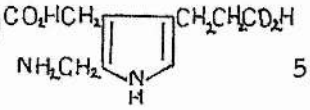
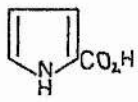
R_4=R_5=H

R_4=H, R_5=OMe

R_4=R_5=OMe

XLVI

Table 5 (cont.)

(k)		558	(l)		535
1, 2, 3-trimethyl			2, 4-dimethyl		
(m)		-	(n)		542
2, 3, 4, 5-tetramethyl			cryptopyrrole		
(o)		558	(p)		498
porphobilinogen			2-carboxylic acid		

The structure of the coloured products formed will now be considered. It is widely assumed that these compounds have structures of the type shown (XXXIII) (60a, 81). Fischer and his co-workers (82) have prepared and identified a number of such compounds but none with only simple alkyl substituents on the pyrrole ring. More recently Morgan and Schunior (83) have prepared a similar compound from pyrrole-2-carboxylic acid. The product (XLIV) from the reaction of 2-methylindole and DMAB in perchloric acid has been isolated and characterised by Dobeneck and Prietzel (28).

Analogous compounds with the structures (XLV, XLVI) have also been prepared (84, 85) using 4-dimethylaminocinnamaldehyde,

4-hydroxybenzaldehyde, vanillin and syringic aldehyde, respectively, in place of DMAB.

A number of products from the pyrroles listed in Table 5 were prepared and an attempt was made to characterise them. They were deeply coloured solids which were soluble only to a slight extent in water and polar organic solvents. Attempts to recrystallise them using a range of solvents and mixed solvents failed to produce crystalline products. Using thin-layer chromatography, the products 'streaked' on the developing plates and a pure sample was unobtainable. An attempt to sublime the products failed, the compounds charring and decomposing before sublimation could take place. Similarly, Castro et al (85) found that the condensation of benzaldehyde, 4-nitrobenzaldehyde and piperonal with cryptopyrrole led only to dark, intractable products. Success in syntheses of this type is, obviously, highly dependent on the groups attached to the pyrrole ring and the condensing aldehyde.

Elemental analyses of the compounds prepared were approximately correct for the proposed structure and in the case of 2-methylpyrrole the mass spectrum showed a small parent peak having a molecular weight in agreement with this analysis. The mass spectra of the other compounds proved difficult to interpret. In dilute acid solution their visible spectra were similar to those obtained under kinetic conditions (see later) but, as in both cases the solutions were not completely stable, complete identification

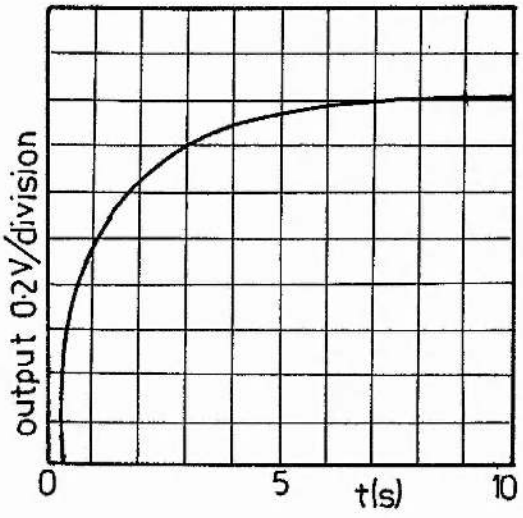


FIGURE 6

Oscilloscope trace of the reaction of DMAB with 1-methylpyrrole in 1·5M-hydrochloric acid.

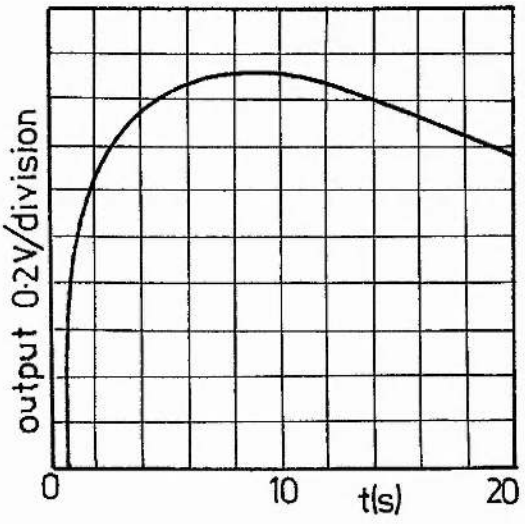


FIGURE 7

Oscilloscope trace of the reaction of DMAB with pyrrole in 1·5M-hydrochloric acid.

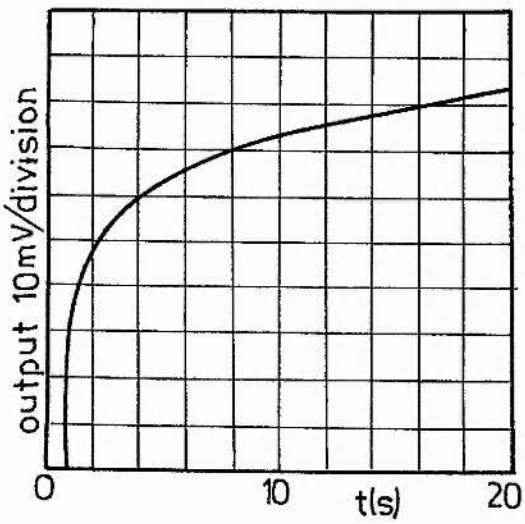


FIGURE 8

Oscilloscope trace of the reaction of DMAB with 2,3,5-trimethylpyrrole in 1·5M-hydrochloric acid

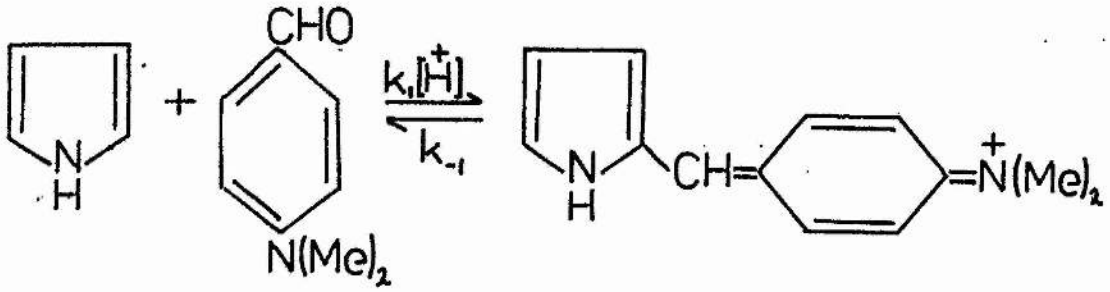
was not possible. The intense absorbance of these compounds is consistent with the long conjugated system of structure (XXXIII) and there seems little doubt that this is the correct structure.

The intense colour of the product formed makes the rate of its formation easy to measure and for all the pyrroles in Table 5 except tetramethylpyrrole and pyrrole-2-carboxylic acid this was carried out using stopped-flow spectrophotometry. Although the measurement of the kinetics is easy, the interpretation of the results is more complex. Broadly, pyrrole compounds fall into three classes with respect to their reaction with DMAB.

(1) The simplest behaviour is good first-order kinetics (DMAB and acid being present in large excesses) with, on a stopped-flow time scale, a constant infinity reading (Figure 6). This behaviour is shown by compounds (b) to (g), (i), and (k) to (o) in Table 5. Over a longer time interval (up to 1 hour) the colour in most cases faded.

(2) With pyrrole itself first-order kinetics are observed initially but fading commences almost immediately and it is impossible to obtain an infinity reading (Figure 7).

(3) 2,3,5-Trimethylpyrrole showed the behaviour illustrated in Figure 8. After an increase in absorbance, which may be first order, the colour intensity increased in a linear manner over several minutes. After that the colour faded, falling to ca. 50% of its maximum value in 1 hour. With this compound attack must be at the 3-position, but similar behaviour is not found with



XXVIII XXIX XXXIII

SCHEME 8

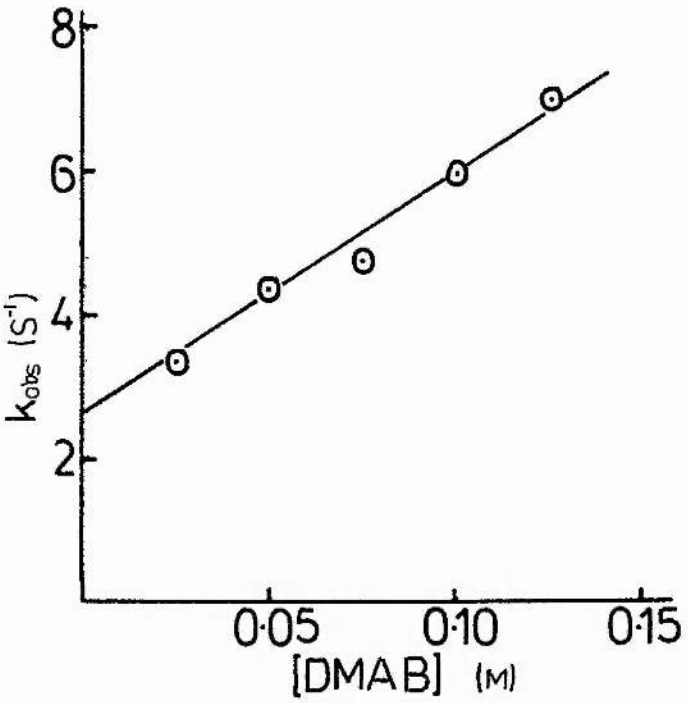


FIGURE 9(a) Variation of k_{obs} with DMAB concentration for reaction with 3,4-dimethylpyrrole in 1·5M hydrochloric acid; [3,4-dimethylpyrrole]$_o = 4 \times 10^{-5}$M

2,5-dimethyl- and 1,2,5-trimethylpyrrole.

The general picture that emerges is that an initial reaction, which obeys first-order kinetics, may be followed by further intensification of the colour, and in the final phase this fades but does not disappear completely. The relative importance of the three phases varies with the pyrrole and this decides if the initial reaction can be separated and analysed satisfactorily. Fortunately, this is the case with most of the pyrroles studied and the only disappointment is that attack at the 3-position could not be analysed due to an insufficient number of results.

In all cases the rate of reaction was determined as a function of DMAB concentration at constant acidity. A plot of the experimentally determined rate constants (k_{obs}) against DMAB concentration was linear in all cases, but with some pyrroles there was a large positive intercept. This shows that the equilibrium in the proposed reaction scheme (Scheme 8) is not completely to the right-hand side. The rate constant (k_{obs}) is given by the equation

$$k_{obs} = k_1 [DMAB] + k_{-1} \qquad (1) ,$$

where k_{-1} includes the hydrogen ion concentration. This equation is derived in Appendix 3 (86) (90).

Absence of an intercept indicates that k_{-1} is zero. The slope of the curve is, of course, k_1 and the results for 3,4-dimethylpyrrole are shown in Figure 9(a). It was found that with the selection of pyrroles studied k_{obs} was independent of the initial pyrrole concentration. The values of k_1 and k_{-1}, together with the relevant pK_a values, are listed in Table 6.

Table 6

Reaction of various pyrroles with DMAB in 1.50 M hydrochloric acid

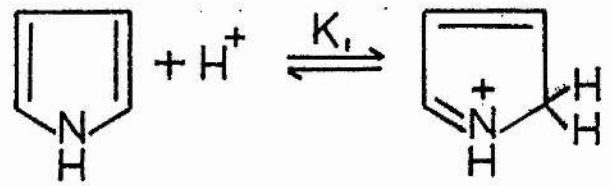
Pyrrole	k_1[a] $mol^{-1}s^{-1}$	k_{-1}[a] s^{-1}	pK_a[b]	kK_2	k_{rel}[c]
a) parent	1.2(±0.3)	0.4	-3.8 (α)	0.41	0.007
b) 1-methyl	5.0(±0.3)	0	-2.9 (α)	1.7	0.03
c) 2,5-dimethyl	<10	4.5	-0.80(α) -0.71(β)		
d) 1,2,5-trimethyl	43(±4)	3.5	-0.21(α) -0.10(β)	40 49	0.80
e) 2-methyl)	17(±2)	0	-0.21(α)	16	0.3
f) 1,2-dimethyl	16(±2)	0	0.50(α)	63	1
g) 3,4-dimethyl	30(±3)	2.8	0.70(α)	161	2.6
i) 2,3-dimethyl	3.7(±0.2)	0	1.5 (α)	120	1.9
j) 2,3,5-trimethyl	<1	0.25	2.0 (α) 0.30(β)		
k) 1,2,3-trimethyl	2.0(±0.2)	0	2.2 (α)	316	5
l) 2,4-dimethyl	2.3(±0.2)	0	2.6 (α)	916	15
m) 2,3,4,5-tetramethyl	0	0	3.7 (α)		
n) cryptopyrrole	0.7(±0.5)	0	3.5 (α)		
p) 2-carboxylic acid	$<10^{-4}$	2.5×10^{-4}			

Notes - a At 25°C

b Except where indicated, the basicity of the β-position is much less than that of the α-position

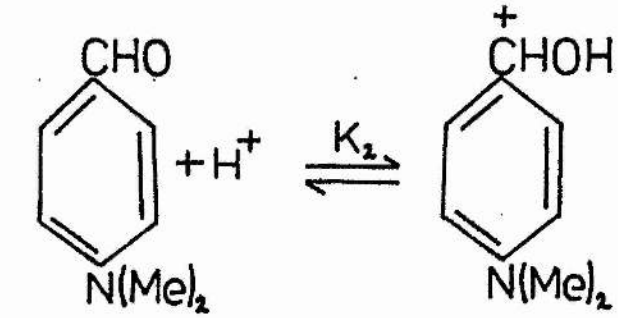
c Rate relative to 1,2-dimethylpyrrole

With compounds (c), (j) and (p) variation of k_{obs} with DMAB concentration was so small that only a very approximate value of k_1 could be determined. For (j), which showed the kinetic behaviour illustrated in Figure 8, the calculation of the first-order rate constant



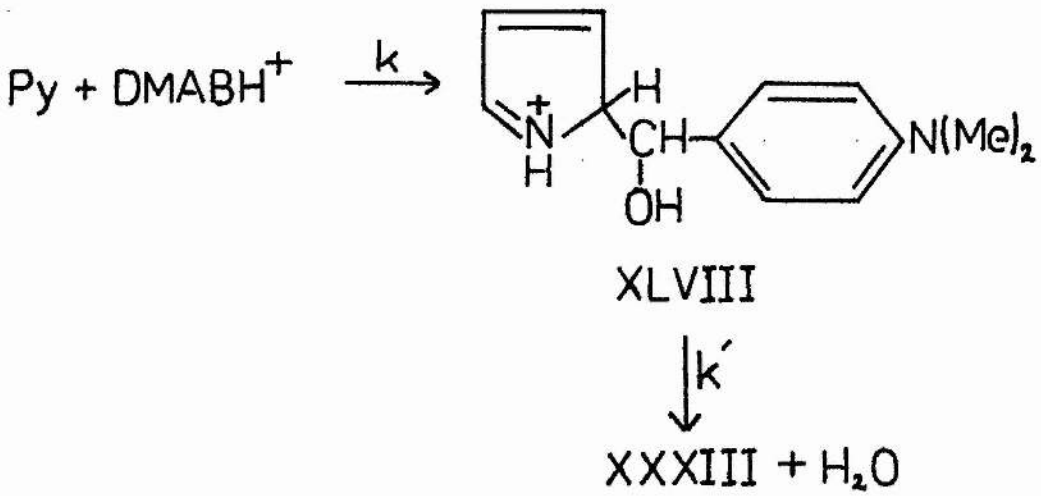
(Py) (PyH+)

XXVIII I



(DMAB) (DMABH+)

XXIX XLVII



XLVIII

XXXIII + H_2O

SCHEME 9

was subject to considerable error and little significance can be attached to the value of k_1. All those pyrroles undergoing attack at the 3-position, (c), (d) and (j), have a substantial back reaction, ie. the equilibrium constant k_1/k_{-1} is small. This is also true of the nonactivated compounds (a) and (p). If the pyrrole is activated towards electrophilic attack a higher concentration of product exists at equilibrium, which is what might be expected. The result for 3,4-dimethylpyrrole is an exception to this behaviour and there is no obvious explanation of this. Indole obeyed good first-order kinetics for each run but a plot of k_{obs} against DMAB concentration showed almost random scatter. A possible explanation of this is given later.

In view of the reaction product and the catalytic effect of acid the reaction mechanism shown in Scheme 9 is proposed. The slow step (k) is attack by O-protonated DMAB on unprotonated pyrrole, and elimination of water (k') is fast. The kinetics are complicated by the fact that pyrroles are protonated to various extents according to the substituents attached to the ring. K_1 is defined as $[PyH^+]/[Py][H^+]$ and the stoichiometric concentration of pyrrole, $[Py]_{st}$, is given by equation 2 and the concentration of the free pyrrole by equation 3.

$$[Py]_{st} = [Py](K_1[H^+] + 1) \qquad (2)$$

$$[Py] = [Py]_{st}/(K_1[H^+] + 1) \qquad (3)$$

K_2 is defined as $[DMABH^+]/[DMAB][H^+]$ and, if the extent of O-protonation of DMAB is small, the rate of the slow step is given by equation 4.

$$\text{rate of formation} = k[Py][DMABH^+] \qquad (4)$$

$$= k[Py]_{st}K_2[DMAB][H^+]/(K_1[H^+] + 1)$$

As the reaction is of the first order in pyrrole the experimentally determined rate constant (k_{obs}), in cases where there is no back reaction, is given by equation 5 and the rate constant k_1, as defined in Scheme 8, by equation 6.

$$k_{obs} = kK_2[DMAB][H^+]/(K_1[H^+] + 1) \qquad (5)$$

$$k_1 = kK_2[H^+]/(K_1[H^+] + 1) \qquad (6)$$

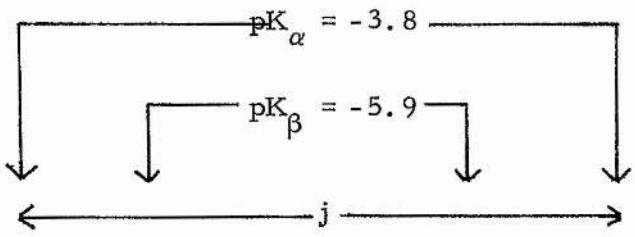
There are four unknowns in the expression for k_1 and the significant one, that which measures the susceptibility of the pyrrole to electrophilic attack, is k. Determination of the other three will now be considered.

The equilibrium constant K_1 is related to the pK_a of the pyrrole. At one time pyrrole was erroneously through to be a moderately strong base (pK_a=0) (17) but Chiang and Whipple (58) have argued that this error was due to a wrong interpretation of the experimental results in which they claim C-protonation was never effected. In turn Chiang and Whipple (58) have made an extensive study of the basicity of a range of methylpyrroles in sulphuric acid using u.v. spectroscopy in which the absorption of the conjugate acid is quite distinct from that of the free base. Using dilute aqueous pyrrole solutions (10^{-4} M) to minimise the chance of polymerisation occurring, they measured the half-protonation point in sulphuric acid as a measure of the pK_a values. The acidities of these solutions were determined using a range of indictor bases. It was found that methyl substituents had an additive effect on the

basicity of the pyrrole ring and from the results obtained they were able to design a table from which the basicity of any methyl substituted pyrrole can be calculated. This is shown as Table 7.

Table 7

$\Delta_m (pK_j)$ values for methyl substitution in pyrroles



m	2	3	4	5
1	0.7	0.8	0.8	0.7
2	-0.6	(3.6)	(1.4)	3.6
3	2.8	(-0.7)	(1.2)	1.7
4	1.7	(1.2)	(-0.7)	2.8
5	3.6	(1.4)	(3.6)	-0.6

notes j position of protonation
m position of methyl substituent
values in brackets represent empirical estimates

Using the experimentally determined pK_a values and the values calculated from the table above the basicities of the pyrroles included in the present study were determined and are included in Table 6.

K_2 is the equilibrium constant for O-protonation of DMAB and is not known, but it is the same in all cases and cancels out in the determination of relative rates. The N-protonated compound, which will be the predominant species, is assumed to be unreactive and its presence does not change the form of equation 6.

The value of $[H^+]$ in equation 6 is not easy to assess. The acid concentration (1.5 M) is outside the ideal range but it is not clear which is the appropriate acidity function to use. (A brief discussion of acidity functions is given in Appendix 4.) The protonation of pyrrole and its methyl derivatives parallels the H_o''' and H_I acidity functions, but log I (where I is the ionisation ratio) is also linear with respect to H_o (58). Protonation of DMAB should parallel the benzophenone scale (H_B) (88) but at low acid concentration H_B and H_o are identical. The h_o value (89) is not, therefore, an unreasonable choice and, in any case, the choice has only a small effect on the relative rates.

It is now possible to calculate the value of kK_2 in equation (6) for most of the pyrroles studied and the values are listed in Table 6.

The ratios of these values gives the relative magnitude of k. The standard was set at unity for 1,2-dimethylpyrrole as this compound gave among the most reproducible kinetics. The spread of rates is about 5,000 but this spread is normally obscured by the effect of protonation.

By comparing the relative reactivities of various pyrroles examined, the activating effect of the methyl group on different positions on the ring can be calculated (Table 8). Where two identical positions are open to attack allowance was made for this.

Table 8

Activating effect of a methyl group towards electrophilic attack on the pyrrole ring

Compounds compared	Position of methyl group	Position of attack	Activating effect
a) and b)	1	2	4.3
e) and f)			3.3
i) and k)			2.6
		Mean value	3.4
a) and e)	2	5	85
b) and f)			67
		Mean value	76
e) and l)	3	2	50
e) and i)	3	5	6.3

Where more than one comparison of the effect of a methyl group on any given position can be made a mean value of the activating effect was determined. The consistency of these results confirms the proposed reaction scheme. It should be noted that as

the pyrrole ring becomes more substituted the activating effect of the methyl group is slightly diminished (see comparisons a) and b), e) and f) and i) and k)). This could mean that the effects are not additive. A more likely explanation, however, is that as the pyrrole ring becomes more substituted steric interactions between the methyl groups and the approaching electrophile become more pronounced.

A similar study to this has been carried out by Butler, Pogorzelec and Shepherd (68) using various diazonium salts as the attacking electrophiles. The activating effects which were derived for the methyl groups (Table 9) compare very favourably with the results listed above although the effect of a methyl group at position 3 on attack at position 5 is smaller than the corresponding value given in Table 8.

Table 9

Activating effect of a methyl group towards electrophilic attack on the pyrrole ring with diazonium ions as the electrophiles

Position of methyl group	Position of attack	Activating effect[a]
1	2	3.2
5	2	49
3	2	48
4	2	1.6

Note a This is the average activating effect calculated for the range of diazonium ions used in the study (68)

The activating effect of a methyl group on pyrrole is less than on thiophen (39, 91 and Chapter 3) but so much confusion

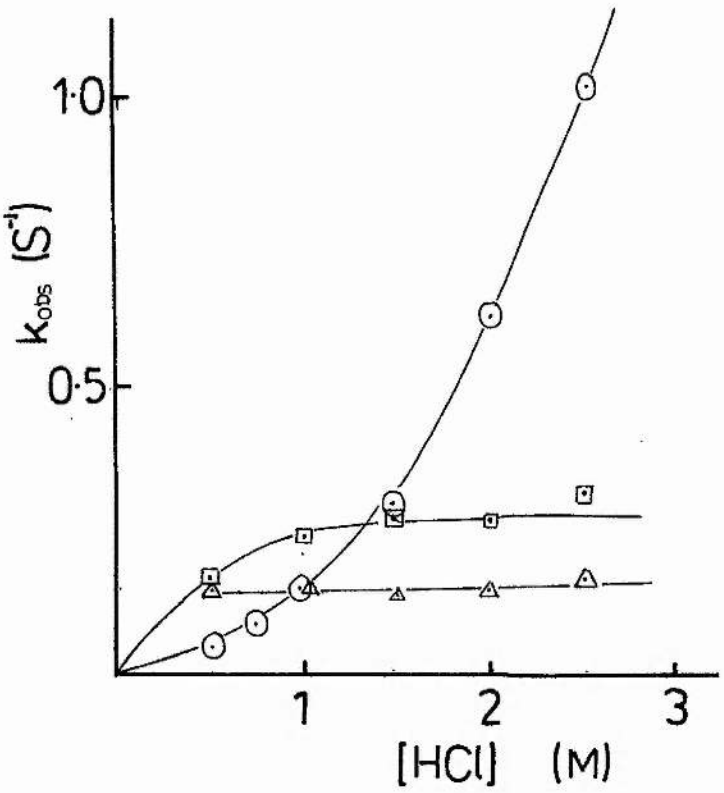


FIGURE 9(b) Variation of k_{obs} with acid concentration for the reaction of DMAB with various pyrroles: ○, 1 methylpyrrole; △, 2,4 dimethylpyrrole; □, 1,2-dimethylpyrrole.

surrounds the meaning of comparisons of substitution effects in different ring systems (92) that it is unwise to speculate on the significance of this observation. The uncertain values of k_1 for compounds (i) and j) makes it impossible to compare the reactivities of the α- and β-positions in this reaction. Compound (d) cannot be used as the effect of 1-substitution on the β-position is unknown.

The validity of Scheme 2 has been further confirmed by a study of the effects of acid concentrations on k_1. If K_1 is very small (ie. the pyrrole is a weak base), then $K_1[H^+]$ is much smaller than unity, equation (6) simplifies to $k_1 = kK_2[H^+]$, and k_1 should be a linear function of $[H^+]$. For 1-methylpyrrole (where K_1 is small) k_1 increases continuously with increasing acid concentration (Figure 9(b)) but the increase is not linear using any known acidity scale. The reason for this discrepancy is not known. When K_1 is large (ie. the pyrrole is a strong base), equation (6) simplifies to $k_1 = \frac{kK_2}{k_1}$ and k_1 should be independent of acidity. This was found to be the case for 2,4-dimethylpyrrole (Figure 9(b)). For a pyrrole of intermediate basicity k_1 should increase initially with increased acidity but become constant at high acid concentration. This was observed with 1,2-dimethylpyrrole (Figure 9(b)).

Two other pyrroles examined showed unexpected behaviour. With 1,2,5-trimethylpyrrole there was the expected increase in k_1 at low acidities but above 1M acid the value decreased (Table 10). The value of k_1 for 2,3-dimethylpyrrole showed a slight but definite decline as the acid concentration was increased (Table 10).

Table 10

Variation of k_1 with acid concentration for 1, 2, 5-trimethylpyrrole and 2, 3-dimethylpyrrole

[HCl] (M)	k_1 ($mol^{-1} sec^{-1}$)	
	1, 2, 5-trimethylpyrrole	2, 3-dimethylpyrrole
0.15	1.5	
0.40	5.8	
0.50		0.13
1.00	7.2	0.13
1.50		0.12
2.00	4.6	0.096
2.50	3.9	0.077

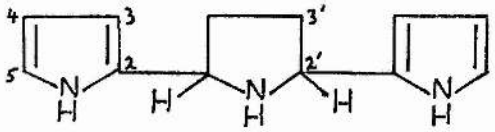
Protonation of indoles shows complex behaviour (93) and differently substituted indoles follow different acidity functions. Similar behaviour with pyrroles might explain these effects, but there is no independent evidence for this.

^{1}H n.m.r. studies by Chiang and Whipple (58) in strong acid indicates that proton loss from the pyrrolinium ion is slow under these conditions. Thus, for N-methylpyrrole in 16 M deuteriosulphuric acid the exchange half-life of a proton at the 2-position is 40 minutes. This could mean that in electrophilic substitution the slow step may be proton loss from the conjugate acid, followed by a fast reaction between the free base and O-protonated DMAB. Very slow proton loss occurs only in very concentrated acid and the half-life of β-protonated 2, 5-dimethylpyrrole in dilute acid is only a fraction of a second (18), but this is still the

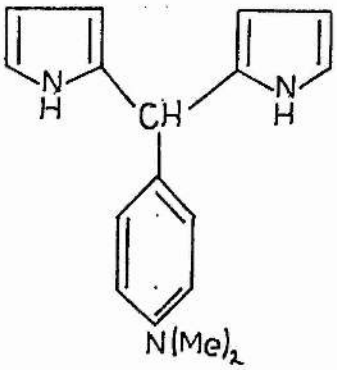
same order of magnitude as the rate of reaction. However, the results in Table 6 argue against proton loss being the slow step. Most of the pyrroles are completely protonated but k_1 varies very little and it is unlikely that the rate of proton loss is unaffected by the large changes in pyrrole basicity. Proton loss does not appear to be the slow step.

The reaction between 2,3,4,5-tetramethylpyrrole and DMAB has been discussed in Chapter 1. It was found that this reaction was several orders of magnitude slower than that of the other pyrroles studied. The matter was not investigated further but the fact that a completely substituted pyrrole gives a positive reaction does not affect the proposed mechanism in Scheme 9.

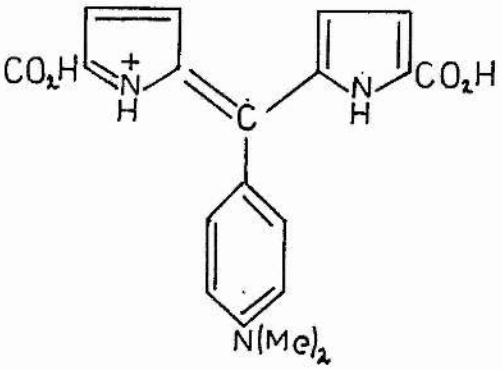
The instability of the product must now be considered and several factors are thought to be important. Firstly, since the Ehrlich reaction is an equilibrium, polymerisation of the pyrroles with time would effectively remove some of the pyrrole available for reaction with DMAB. The more basic pyrroles, which are completely protonated in acid will not polymerise under these conditions, display fairly straightforward kinetics. On the other hand, pyrrole, being weakly basic, polymerises readily in 6M hydrochloric acid, the product being the trimer (XIII). A sample of this compound was prepared and reacted with Ehrlich's reagent. The resultant u.v. spectrum showed an absorption at 552 nm with a shoulder at 525 nm (pyrrole with DMAB gives an absorption at 560 nm) and this may explain why the colour does not fade completely.



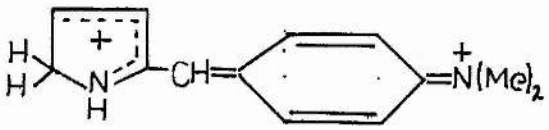
XIII



XLIX



L



LI

The ^{13}C n.m.r. spectrum of the trimer was taken and showed six peaks at 32.66, 55.44, 104.28, 108.38, 116.93 and 134.65 ppm relative to a TMS internal reference. The two high field absorptions, 32.66 and 55.44 ppm, are due to the 2'- and 3'-carbon atoms (XIII) respectively of the pyrrolidine moiety. These can be compared with the corresponding carbon absorptions of unsubstituted pyrrolidine at 25.7 and 47.1 ppm (94). The low field absorptions at 104.28, 108.38, 116.93 and 134.65 ppm can be assigned to carbons 3, 4, 5 and 2 (XIII) respectively of the pyrrole moiety. This is based on the study of ^{13}C- n.m.r. spectra of pyrroles by Abraham et al (59) and assumes that the pyrrolidine substituent has a similar effect to that of a methyl group on the chemical shifts of the pyrrole ring-carbons, although of a different magnitude.

As reported above the kinetics of the reaction between indole and DMAB are random and impossible to interpret. The product from this reaction was obtained and contained, from mass spectral and microanalytical evidence, two indolyl groups for each DMAB. Under acid conditions, therefore, indole probably dimerises (95) before it reacts and this could explain the above kinetic results. Dimerisation and trimerisation of indole are known (87, 96) to occur readily in aqueous acid and the resulting solution contains an equilibrium mixture of indole, its dimer, and trimer and their salts (97).

Treibs and Herrman (60a) have suggested that formation of a dipyrrylphenylmethane (XLIX), which is colourless, may

account for the fading. However, Morgan and Schunior (83) have isolated the coloured compound (L) from the reaction of the initial Ehrlich product with a further mole of pyrrole-2-carboxylic acid. Further evidence exists to cast doubts on the above two explanations of colour fading. Firstly, the pyrrole concentration is so small (10^{-4} M) under the kinetic conditions used that it seems unlikely that polymerisation could occur and, secondly, with such a large excess of DMAB over pyrrole it is very unlikely that any unreacted pyrrole would be available to undergo further reaction with the initial Ehrlich product to give compounds such as (XLIX).

A more likely explanation can be found, however, in the formation of the simple, colourless, di-protonated salt (LI) (98) of the initial product, formed under acid conditions. The formation of this compound does not suffer from any of the drawbacks mentioned above and under the acid conditions found in the kinetic studies its formation may be expected.

Experimental.-

Materials.-

Pyrrole, N-methylpyrrole, 2,5-dimethylpyrrole, cryptopyrrole, pyrrole-2-carboxylic acid, porphobilinogen and indole were obtained commercially. 2-Methyl- (61), 2,3-dimethyl- (61), 2,3,5-trimethyl- (61), 2,4-dimethyl- (99) and 2,3,4,5-tetramethylpyrrole (62) were prepared by known methods. Preparation of 3,4-dimethylpyrrole by the method of Stapfer and D'Andrea (100) was unsuccessful but the compound was readily obtained by another published route (61). The N-methylated pyrroles were prepared by reaction of the appropriate methylpyrrole with potassium and iodomethane (61). A sample of 1,3,4-trimethylpyrrole was also obtained using the method of Chapelle et al (80). Pyrrole trimer was prepared by the method of Potts and Smith (23). 4-Dimethylaminobenzaldehyde and hydrochloric acid were AnalaR grade.

Kinetics.-

The pyrroles were distilled or recrystallised where appropriate prior to use. The spectra of the coloured products were recorded on a Unicam SP800 spectrometer.

A 'Canterbury' stopped-flow spectrophotometer was used for most of the kinetic studies. A very dilute solution (10^{-4} M) of pyrrole in distilled water was placed in one arm and DMAB in standard HCl (0.25 M DMAB in 3 M HCl) in the other. The oscilloscope trace obtained on mixing was photographed. The

wavelengths used for the various pyrroles were those listed in Table 5. The slow reaction of pyrrole-2-carboxylic acid was examined on a Unicam SP500 spectrophotometer. As constant infinity readings were not attainable, the observed rate constants were calculated by the method of Swinbourne (65) and Kezdy (64) (Appendix 2).

Products.-

DMAB (1.5 g) was dissolved in carbon tetrachloride (30 ml) and to this was added water (150 ml) containing 2 drops of concentrated HCl and pyrrole (0.8 g) dissolved in carbon tetrachloride (15 ml). The mixture was refluxed for 3 hours, cooled, the water layer separated and washed with ether (3 x 25 ml). The salt was obtained as a dark solid by evaporation of the aqueous solution.

N.m.r. studies.-

The ^{13}C n.m.r. spectrum of pyrrole trimer was taken with chloroform as solvent and an internal reference of TMS.

Chapter 3

Acid-catalysed hydrogen-exchange in methylthiophens

Introduction.-

Thiophen, like pyrrole, undergoes facile electrophilic substitution and in recent years these substitution reactions have been the subject of numerous kinetic and mechanistic studies. Thus, halogenation (101), acylation and related reactions (101e, 102), hydrogen exchange (103), replacement reactions (104), nitration (105) and mercuration (105b) have all been studied quantitatively.

In most cases, substitution on the thiophen ring proceeds by a similar mechanism to that for the analogous reaction of benzene derivatives. The case of hydrogen-exchange, on which extensive kinetic studies have been based, will be discussed later. One of the few exceptions to this general similarity of reaction mechanisms is found in mercuration of thiophen, where preliminary coordination of mercury with the sulphur atom is thought to occur (106).

In all the electrophilic substitution reactions for which data are available, thiophen is much more reactive than benzene; its reactivity being generally comparable to that of anisole. Thus, thiophen reacts 1.7×10^9 times faster than benzene in bromination (101a), 1.3×10^7 in chlorination (101a), 9×10^5 in acylation (101e) and from 50 to 1250 times (according to the conditions) in nitration (105d, e, f).

On comparing the reactivity of thiophen with other five-membered heteroaromatic systems containing one heteroatom, all existing data show that it is the least reactive towards electrophilic attack of the series: pyrrole $>$furan $>$tellurophen $>$selenophen $>$thiophen (101c, 102b).

It is well known that electrophilic attack leads to a predominance of 2- as opposed to 3-substituted products. The preference for 2-substitution can be rationalised by comparing the energies of the proposed transition states leading to 2- and 3-substituted products; taking the 'Wheland' intermediates as models for the transition states it is possible to write three limiting resonance structures for the 2-attack and only two for the 3-attack. (These structures are essentially the same as those given for pyrrole in the General Introduction.) It is also found that the isomer distribution of products is dependent on the nature of the electrophile; in general the 2:3 ratio is greater when the electrophile is milder. On comparing this property of thiophen with that of other five-membered heterocylic ring systems, although data for a homogenous comparison under strictly equivalent conditions are not yet available, all existing data seem to confirm the general validity of the following order for the 2-orientating power:

$$\text{furan} > \text{thiophen} \gg \text{pyrrole}.$$

In the case of hydrogen-exchange in thiophens, where extensive data are available (103), it is also found that the rate of reaction at the 2-position is much greater than that at the 3-position. This is in contrast to the situation found in pyrrole where the exchange rate at both positions is similar.

It is interesting to compare these experimental results with the predicted reactivity of thiophen based on theoretical studies. Two recent theoretical studies have been undertaken (107, 108) and the values for the Free Valence Index, the

Superdelocalisability and the Wheland's Localisation Energy derived by Klasinc and Humski (107) are given in Table 11.

Table 11

Reactivity indices of thiophen

Position	Free valence index (Fr)	Superdelocalisability (Sr)	Wheland's Localisation Energy ($L^{+}r$)
2	0.523	1.364	1.734
3	0.391	1.046	2.289

The Free Valence Index is closely related to the π-electron density associated with atom r of the ring whereas the Superdelocalisability is derived by application of perturbation theory to a model in which the incoming group forms a weak π-bond to atom r of an otherwise unmodified π-system. The Wheland's Localisation Energy is the π-bonding energy required to isolate two electrons at position r from the remainder of the π-network.

Marino (109) has argued that the high reactivity of thiophen, as compared with benzene, towards electrophiles does not depend on a high electron density on the carbon atoms in the ground state. This he bases on the fact that the dipole moment in thiophen is directed from the ring to the heteroatom (109) and the experimental observation that thiophen compounds are more reactive than the corresponding benzene derivatives towards nucleophilic substitution (110). The difference in the value of the Free Valence Index for

the 2-position of thiophen (Table 11) and the value of 0.399 for benzene (111a) is, however, significant and it would seem reasonable to expect that this difference would account, in part, for the greater reactivity of thiophen. The fact that the dipole moment of thiophen is directed towards the heteroatom may simply be due to the high electron density associated with the sulphur atom. The low localisation energy of thiophen (Table 11) as compared to that of benzene (2.536) (111b) is also consistent with the greater reactivity of the former.

The reactivity indices given in Table 11 are all consistent with the fact that the main product of electrophilic attack on thiophen is the 2-substituted compound and that the rate of substitution is greater at the 2-position than at the 3-position.

These findings can be compared with the corresponding results for pyrrole where 2-substituted products predominate but the rates of electrophilic substitution at the 2- and 3-positions are similar (Chapter 1). The properties of pyrrole pertinent to this discussion are given in Table 12.

Table 12

Charge densities and localisation energies for pyrrole

Position	Charge Densities				Localisation Energy	
	a	b	c	d	L^{+}_{r}	
1	0.382	0.436	0.364	-0.10		
2	-0.090	-0.102	-0.072	0.07	1.4844	b
3	-0.101	-0.116	-0.110	-0.03	1.6412	b

a ref. 112 Huckel method
b ref. 113 Huckel method
c ref. 114 SCF method
d ref. 33 INDO method

The localisation energies for pyrrole are consistent with the fact that 2-substituted products predominate and the fact that they are smaller than the corresponding values for thiophen (Table 11) is in agreement with the greater reactivity of pyrrole than thiophen towards electrophilic substitution. Although the absolute values for the charge densities round the pyrrole ring depend on the method of calculation (Table 12) the important feature is that the β-carbon is slightly more electronegative than the α-carbon. This is in contrast to the situation found in thiophen where the α-carbon is the more electron-rich. This difference can be used to explain why the α:β reaction rate ratio for pyrrole is so different from that found in thiophen. Thus, as an electrophile approaches either of these compounds it will be attracted to the site of highest negative charge which in the case of thiophen is the 2-position whereas in pyrrole the 3-position is the slightly more negative. The fact that reaction rates are approximately equal for the two positions in pyrrole (at least in the case of hydrogen-exchange) has been discussed earlier and is probably due to the fact that bond bending within the pyrrole ring can reverse the order of electronegativity found at the α- and β-carbons.

In conclusion, therefore, both the predominant product formed from, and the relative rate of, electrophilic substitution on these ring systems can be predicted using the reactivity indices considered above. Thus, the product ratio found at equilibrium

is wholly dependent on the thermodynamics of the system and is best monitored by the localisation energies of the various positions on the rings. However, the reaction rates at these positions, which depend on the rate of attack of the electrophile, are best understood in terms of the Free Valence Index (or electronegativity), a property of the ground state molecule.

Results and discussion.-

This study represents an attempt to determine the quantitative effect of methyl groups on the kinetics of hydrogen-exchange in thiophens in acidic media. The thiophens used were 2-methyl-, 3-methyl-, 2,3-dimethyl-, 2,4-dimethyl- and 2,5-dimethylthiophen. Due to experimental difficulties no attempt was made to measure the rates of exchange at two positions of similar reactivity in the same molecule. Thus, for 2-methylthiophen, where the 3- and 4-positions are chemically different but similar in reactivity, only exchange at the 5-position was measured. The fact that the reactivities of the 2- and 3-positions of thiophen are so very different made it easy to study exchange at either position, to the exclusion of the other, by varying the acid concentration used.

The exchange rates obtained for the 2-position in 0.085 M perchloric acid are given in Table 13. Extrapolation of the results of Butler and Hendry (103f) to 0.085 M acid gives a value of k_{obs} for protodetritiation of 2-tritiothiophen of 1.15×10^{-7} s^{-1} at 25°C. This figure, combined with the results in Table 13, gives the activating effect of a methyl group at positions 3, 4 and 5 on the rate of exchange at the 2-position (Table 14).

The extrapolation of the results of Butler and Hendry (103f) is a long one and so the value in Table 14 for the activating effect of the 5-methyl group is subject to uncertainty. There is also a small error due to the difference in temperature of the two

Table 13

Protodetritiation of substituted 2-tritiothiophen in 0.085 M aqueous perchloric acid

	Substituent (s)	Temperature (^{o}C)	$10^{5} k_{obs}$ (s^{-1})
(a)	5-methyl	27.0	2.23
		35.2	4.62
		45.3	9.36
		55.2	18.4
(b)	4,5-dimethyl (2,3-dimethyl)	27.3	2.63
		35.2	5.10
		45.0	10.8
		55.1	23.3
(c)	3,5-dimethyl) (2,4-dimethyl)	27.3	65.3
		35.2	108
		45.0	212
		55.1	444

Table 14

Activating effect of a methyl group towards hydrogen-exchange at the 2-position of the thiophen ring at 27^{o}C

Compounds compared	Position of protodetritiation	Position of methyl group	Activating effect
thiophen and (a)	2	5	194
(a) and (b)	2	4	1.2
(a) and (c)	2	3	29

determinations. However, the value obtained agrees well with that (ca. 200) determined by Butler and Eaborn (103g) for protodetritiation in trifluoroacetic acid.

Compounds (b) and (c) contain two methyl groups and the effect of the second methyl group on the rate of protodetritiation is more difficult to understand. The effect of a methyl group at the 'diagonal' position in (b) is small, but this is not unexpected as there is no resonance effect in the 'Wheland' intermediate for a methyl group at that position. The effect of an extra methyl group at the 3-position in (c) on exchange at the 2-position is surprisingly small. Shatenshtein et al (115) report that 3-methyl-2-tritiothiophen protodetritiates in trifluoroacetic acid 340 times faster than 2-tritiothiophen. This may be compared with the activating effect of a 5-methyl group of 194 reported by Butler and Eaborn (103g). Thus, it would be expected that a methyl group at the 3-position would have a similar effect to that of a 5-methyl group. However, the present results indicate that, with both methyl groups present in the molecule, their effects on protodetritiation are not additive.

There is no evidence from previous work that, in thiophen compounds, the effects of two methyl groups are additive. Ansell and Taylor (116) found that activating effects were not additive in the protodetritiation of tritiated xylenes. Katritzky et al (117) have reported similar behaviour with a number of 5-membered aromatic heterocycles. However, these effects are smaller than that observed in the present work. A more direct comparison can be made with the work of Shatenshtein et al (118) on the

protodedeuteration of deuteriated phenylthiophenes. For exchange at the 2-position both 5- and 3-phenyl groups activate the reaction to comparable extents. On the other hand, with 4, 5-diphenylthiophen exchange is actually slightly slower than with 5-phenylthiophen, a result which is in agreement with the above result for 4, 5-dimethylthiophen. However, these workers found that in 3, 5-diphenylthiophen the effect of the phenyl groups is additive. The result given above for 3, 5-dimethylthiophen is not in agreement with this observation and there is no obvious explanation for this. The value for the 5-methyl group, owing to the long extrapolation, is subject to considerable uncertainty and the difference between the results given in Table 14 and those of Shatenshtein may be less than the figures suggest. On the other hand it may be greater.

From the results recorded in Table 13 activation parameters for protodetritiation at the 2-position have been calculated (Table 15) (Appendix 5).

Table 15

Activation parameters for the protodetritiation of methylthiophens at 40°C

Compound	$[HClO_4]$ (M)	Position of protodetritiation	$\Delta H^{\ddagger}$ (Kcal mol^{-1})	$\Delta S^{\ddagger}$ (cal $mol^{-1}K^{-1}$)
(a)	0.085	2	14.0(±0.3)	-33 (±2)
(b)	0.085	2	14.6(±0.3)	-31 (±2)
(c)	0.085	2	13.6(±0.5)	-28 (±2)
(d)	3.95	3	16.4(±0.5)	-21 (±2)
(e)	3.95	3	16.0(±0.4)	-22 (±2)
(f)	3.95	3	15.5(±0.5)	-23 (±2)
(g)	3.95	3(4)	13.4(±0.4)	-31 (±2)

Notes: for compounds (a), (b) and (c) see Table 13
for compounds (d), (e), (f) and (g) see Table 16

Changes in the rate of reaction are due to changes in the enthalpy of activation and, as far as can be judged from the limited data, the entropy of activation remains constant at ca. 31 cal $mol^{-1}K^{-1}$. This value is very different from that measured by Butler and Hendry (103f) for protodetritiation of thiophen in concentrated sulphuric acid (14.6 cal $mol^{-1}K^{-1}$). This is probably due to differences in hydration of the proton in the two media.

Hydrogen exchange at the 3-position of thiophen is much slower than at the 2-position and results for the protodetritiation of substituted 3-tritiothiophen in 3.95 M perchloric acid are given in Table 16.

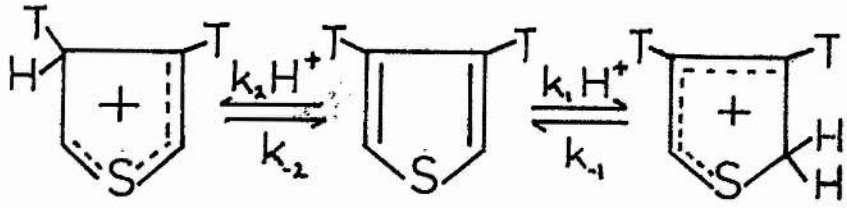
Table 16

Protodetritiation of substituted 3-tritiothiophen in 3.95 M aqueous perchloric acid

Substituent(s)	Temperature (°C)	$10^3 k_{obs}$ (S^{-1})
(d) 4-methyl (3-methyl)	27.0	0.166
	35.2	0.311
	45.3	1.02
	55.2	1.86
(e) 4,5-dimethyl (2,3-dimethyl)	27.0	0.280
	35.2	0.643
	45.5	1.31
	55.0	2.94
(f) 2,4-dimethyl	27.0	0.282
	35.2	0.491
	45.3	1.29
	55.2	2.24
(g) 2,5-dimethyl	27.0	0.154
	35.2	0.286
	45.5	0.566
	55.0	0.966

Exchange with unsubstituted 3-tritiothiophen in this acid was found to be too slow to determine and so there is no measure of the effect of a single methyl group at the 4-position. However, comparison of the values of k_{obs} for (e) and (f) with those for (d) shows that introduction of a second methyl group, wherever it is located, has very little effect on the rate. For a 5-methyl group this is reasonable as it is diagonally situated with respect to the site of reaction. A much larger effect is anticipated for a methyl group at the 2-position, as in (f), as here the substituent is ortho to the site of reaction. Even if the effect of a second methyl group is not additive, the activating effect of that group is still surprisingly small. It is much less than the effect of a 3-methyl group on exchange at the 2-position. There is an explanation for this, however. One distinctive feature of the thiophen ring is the large difference in reactivities of the 2- and 3-positions, much larger than for pyrrole. If introduction of a methyl substituent lowers the activation energy of reaction by a similar amount, then a methyl group will affect the reaction rate at the 2-position much more than at the 3-position. The substituent effect for compound (f) is consistent with this analysis.

One other effect of methyl substitution on the thiophen ring should be considered here. In pyrrole chemistry it is found that methyl groups attached to the pyrrole ring increase the basicity of the pyrrole considerably and should a similar situation occur in thiophen chemistry this could affect the kinetics of hydrogen exchange. Thus, in the study of hydrogen-exchange at the 3-position of thiophen,



LIII LII LIV

followed by protodetritiation

cannot undergo protodetritiation

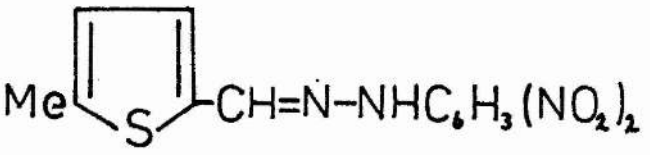
SCHEME 10

allowance would have to be made for any protonation at the 2-position which would render some of the tritiothiophen unreactive (Scheme 10). Introduction of a methyl group into the 2-position of the thiophen ring, for example, may increase the basicity of the 5- and 3-position in an analogous way to that found in pyrrole. This would mean that the value of k_2 would be increased and subsequently an increase in the rate of protodetritiation would be expected. However, the value of k_1 would also be increased and therefore a higher proportion of the tritiothiophen would be in the unreactive 2-protonated form. If the introduction of the methyl group has the same effect on k_1 as on k_2 then no overall increase in the rate of protodetritiation may be observed. This could explain the results given in Table 16. However, the only evidence from the literature that thiophens protonate to any appreciable extent is found in the work of Hogeveen (119) where the n.m.r. spectrum in HF at $-60^{\circ}C$ of the 2-protonated species is recorded. An attempt was made, therefore, to determine if protonation occurs under less extreme experimental conditions by recording the u.v. spectra of several methylthiophens in a range of aqueous sulphuric acid mixtures (10% $\longrightarrow$ 70% H_2SO_4). Should protonation occur, a change in the spectra similar to that observed by Chiang and Whipple (58) for pyrroles would be expected. However, on comparing the spectra of the thiophens recorded in distilled water and in the acid solutions above, no significant changes were noted. This, presumably, means that no significant protonation occurs under these conditions

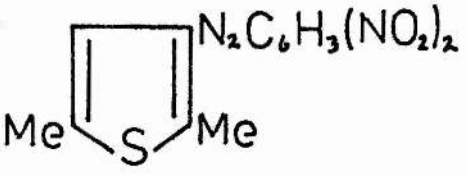
and therefore no allowance need be made for it in the present study.

Activation parameters for protodetritiation at the 3-position have been calculated from the data in Table 16 and are given in Table 15 (Appendix 5). The difference in the reactivity of the 2-3-positions is caused by changes in both the enthalpy and entropy of activation. The situation is complicated by the fact the parameters were determined at different acidities and, as has already been noted, this may cause a change in the entropy of activation. The activating parameters for protodetritiation of 2,5-dimethyl-3-tritiothiophen are significantly different from those for exchange at the 3-position with compounds (d), (e) and (f). There is no obvious explanation for this. The more negative value of $\Delta S^{\ddagger}$ for (g) could indicate a change in mechanism, but it is difficult to see what this change could be.

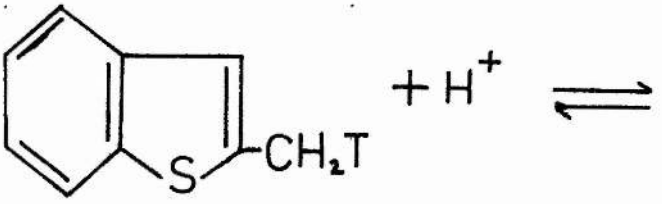
A comparison between the transmission of substituent effects in thiophen and pyrrole with respect to electrophilic substitution can now be made. The latter situation has been discussed in Chapter 2 and a similar study has been made by Butler et al (68) on the reaction between methylpyrroles and various diazonium salts. In the pyrrole reaction discussed in Chapter 2 a methyl group at the 5-position has a smaller effect on reaction at the 2-position than in protodetritiation of 2-tritiothiophen. The results in Table 14 would tend to indicate that the effect of a 3-methyl group on protodetritiation at the



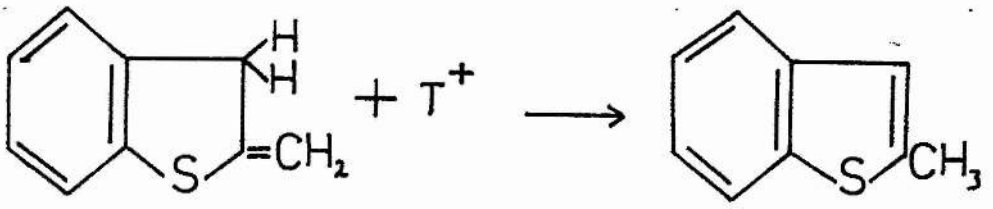
LV



LVI



LVII



LVIII LIX

SCHEME 11

2-position is less than that of a 5-methyl group. However, this is probably due to non-additivity of methyl groups in polymethylthiophens discussed previously. If the value of 340 for the activating effect of a 3-methyl group reported by Shatenshtein (115) is taken as being more 'realistic' then this is comparable with the value of 194 given in Table 14 for the activating effect of a 5-methyl group. This is a similar situation to that found for pyrrole, where the activating effects of a 5-methyl and a 3-methyl group on reaction at the 2-position are similar in magnitude. It should be noted that the magnitude of these activating effects found in the thiophen ring are greater than the corresponding effects in the pyrrole ring.

In a study of the reaction between various polymethylthiophens and 2,4-dinitrophenyldiazonium ions Tedder et al (56) found that, as well as the expected ring attack, there may be reaction with a methyl group. For example, 2,5-dimethylthiophen reacts to give (LV) as well as (LVI). Reaction with a methyl group does not occur with less activated benzenediazonium ions. Electrophilic substitution at methyl groups attached to aromatic systems is not uncommon. Kharkharov (50) has reported coupling of 4-nitrobenzenediazonium ions with the methyl group in 2,4,6-trinitrotoluene and more recently a report of the coupling of 2,3-dimethylindole with 2-methyl-4-nitrobenzenediazonium ions has appeared in the literature (54). Electrophilic substitution at the 2-methyl group of 2,3-dimethylthianophthalen has been reported by Bordwell (51) and electrophilic attack on methyl groups attached to hexamethylbenzene and polyalkylnaphthalenes has also

been described (52). The specific case of hydrogen-exchange reactions at active methyl groups of heterocyclics has been reviewed by Bologa and Gard (120). More recently, Parham and Olsen (55) have reported hydrogen exchange reactions at the methyl groups of methylpyridines and Eaborn and Wright (53) have made a study of exchange reactions at the methyl group of 2-methylbenzothiophen.

Eaborn and Wright (53) have proposed the mechanism shown in Scheme 11 for methyl group hydrogen-exchange and a similar mechanism could explain the formation of (L.V). They also report that, for 2-methylbenzothiophen, ring exchange is at least 2000 times faster than exchange at the methyl group, whereas for the reaction of 2,5-dimethylthiophen with 2,4-dinitrobenzenediazonium ions (56) the yields of products resulting from ring and methyl group attack were roughly equal. Two important considerations should be noted, however. Firstly, the overall yield of the reaction between 2,5-dimethylthiophen and the diazonium salt was only 27% and secondly, as noted in Chapter 1, the ratio of products obtained at equilibrium in a synthetic preparation is not necessarily related to the rate of electrophilic attack leading to these products. It was decided, however, to examine the rate of hydrogen-exchange at the methyl groups and compare this with the exchange rate found at the ring of 2,5-dimethylthiophen. For exchange of the methyl group hydrogens k_{obs} in 3.95 M perchloric acid was found to be $1.29 \times 10^{-5} s^{-1}$, only 45 times slower than exchange of a ring proton in the same acid. Therefore, the very large difference in

the reactivity of ring and methyl group protons, observed with 2-methylbenzothiophen, does not apply to 2,5-dimethylthiophen.

Tedder et al (56) also measured hydrogen exchange at the methyl groups of a number of methylthiophens by examining changes in the ^{1}H n.m.r. spectra with time when the components were dissolved in deuteriotrifluoroacetic acid. They observed immediate exchange of all the ring protons followed by much slower exchange of the protons in certain methyl groups. Gore (121) reports that exchange of the methyl group protons of 2,5-dimethyl- and 2,4-dimethylthiophen is very slow and incomplete after several hours. This result appears to confirm the large difference in reactivity of ring and methyl group protons reported by Eaborn and Wright.

The work of Gore (121) has been repeated and the results interpreted in the light of the present studies of the kinetics of hydrogen-exchange. These results are shown in Table 17. With 2,4-dimethylthiophen in deuteriotrifluoroacetic acid exchange of the ring protons was complete before the first n.m.r. spectrum could be recorded. Over the next 6 hours there was gradual exchange of the protons of the 2-methyl group, but reaction ceased when about half the protons had exchanged. There was no exchange at the 4-methyl group. The results given elsewhere would indicate that the high acidity of neat trifluoroacetic acid (122) should lead to very rapid exchange of the ring protons in both the 3- and 5-positions. However, exchange of the methyl group protons

would also have been expected to be complete within the period of the experiment. The fact that this does not occur can be explained in terms of the experimental conditions. In order to obtain n.m.r. spectra the initial concentration of 2,4-dimethylthiophen, which contains five exchangeable hydrogens (trifluoroacetic acid contains only one exchangeable hydrogen), was ca. 0.9 M. As exchange proceeded the deuteriotrifluoroacetic acid contained increasing amounts of protium and the final solution was an equilibrium one. The apparent decrease in the rate of deuteriodeprotonation of the 2-methyl group was due to the kinetics of the approach to equilibrium.

Similar results were obtained with 2-methylthiophen. About 50% deuteriodeprotonation of the methyl group occurred during 6 hours. With 3-methylthiophen no exchange at the methyl group occurred and this is consistent with the fact that no exchange occurred at the 4-methyl group of 2,4-dimethylthiophen. With 2,3-dimethylthiophen decomposition of the sample took place during the course of the reaction.

Table 17

^{1}H n.m.r. spectra of methylthiophens taken in deuteriotrifluoroacetic acid

Compound	Time (hr)	$MeCl_2$/methyl thiophen ratio[b]		Relative ratio[c]	
		2.2	2.5	2.2	2.5
2,4-dimethyl	O($CDCl_3$)[a]	1.13	1.02	1	1
	0.5	1.08	1.14	1	1.12
	1.5	1.08	1.39	1	1.36
	4	1.10	1.85	1	1.81
	6.5	1.14	2.02	1	2.01
			2.5		
2-methyl	O($CDCl_3$)[a]		0.78		1
	0.5		0.85		1.09
	2		1.23		1.58
	4.5		1.40		1.79
	6.5		1.62		2.07
			2.3		
3-methyl	O($CDCl_3$)[a]		1.00		1
	0.5		0.97		1
	1.5		0.97		1
	4		0.95		1
	7		0.98		1
2,3-dimethyl	O($CDCl_3$)[a]				
	0.5				
	1.5	Decomposition of sample occurred			
	3.5				
	6				

Notes a - results refer to ^{1}H n.m.r. spectrum taken in $CDCl_3$ for the same $MeCl_2$:thiophen mixture

b - the values correspond to the methyl group resonances, with reference to TMS, in $CDCl_3$

c - calculated assuming ratio at zero time being unity

Experimental.-

Materials.-

2-Methyl-, 3-methyl- and 2,5-dimethylthiophen were purchased from Aldrich Chemical Company. 2,3-Dimethylthiophen was prepared by a literature method (123) and 2,4-dimethylthiophen was a gift from Professor J.M. Tedder. Deuteriotrifluoroacetic and tritiotrifluoroacetic acids were prepared by reaction of trifluoroacetic anhydride with deuterium oxide and tritiated water, respectively. The perchloric acid used was AnalaR grade.

Thiophens tritiated at all the ring positions were prepared by stirring the appropriate compounds (0.5 g) overnight with a mixture of 60% H_2SO_4 (1 ml) and tritiated water (1 ml; 50 mCi/ml). The resultant mixture was neutralised with an excess of NaOH and extracted with ether (50 ml). After washing with distilled water (2 x 25 ml), the organic extracts were dried ($MgSO_4$) and the solvent removed by evaporation. Tritiodeprotonation of the methyl groups of 2,5-dimethylthiophen was effected by stirring the thiophen with tritiotrifluoroacetic acid overnight. The tritiated thiophens were distilled before use.

Kinetics.-

An aqueous solution of the required thiophen was prepared by adding a drop of the tritiated thiophen to 100 ml of distilled water, stirring overnight and filtering the solution through phase separating paper to remove any undissolved thiophen. The purity of these solutions was monitored by recording their u.v. spectra.

The method for obtaining the kinetic data was similar to that described in Chapter 1 for hydrogen-exchange in pyrroles.

Exchange at different positions in the thiophen ring occurs at very different rates and the reactions do not interfere with one another. For example, in the acid used to study exchange at the 3-position, exchange at the 2-position is so rapid that it is complete within a few minutes. On the other hand, in 0.085 M perchloric acid essentially no protodetrititiation occurs at the 3-position within the time interval used to study exchange at the 2-position. In the case of methyl group hydrogen-exchange in 2,5-dimethylthiophen a sufficiently long time interval, to ensure that all ring protons had exchanged, was allowed to pass before taking the first measurement.

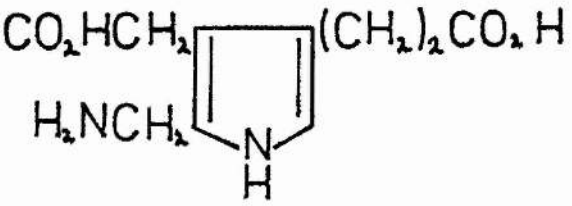
The rate constants were calculated using the method of Swinbourne (65) and Kezdy (64) (See Appendix 2).

N.m.r. studies.-

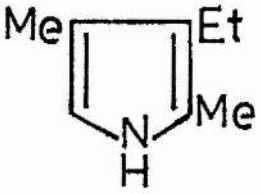
A solution of the thiophen in deuteriotrifluoroacetic acid (0.9 M), with an equal amount of dichloromethane as a standard, was prepared and spectra were recorded at timed intervals. Where the thiophen had a methyl group which did not undergo exchange, its signal remained constant with reference to that of dichloromethane and showed that the thiophen was stable in the acid during the time of the experiment.

Chapter 4

The detection and quantitative determination of the clinically important pyrroles porphobilinogen and cryptopyrrole



XXXIV



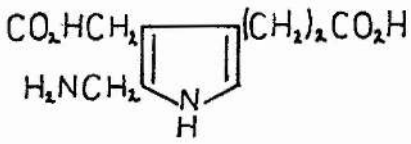
XXXVI

Introduction.-

As noted in Chapter 2 the determination of clinically and biologically important pyrrole-containing compounds is well established. This present work is a study of the practical difficulties encountered in the quantitative determination of two of these pyrroles, namely porphobilinogen (XXXIV) and cryptopyrrole (XXXVI). The main difficulties involved are threefold. Firstly, the substance being estimated must be separated from any other components present in the biological sample which may interfere with the subsequent test and, secondly, the solution being tested should be stable. Both these problems have, at least in part, been overcome. Thus, in the case of porphobilinogen, this compound can be separated from the other main components found in urine, for example, by means of column chromatography (124), the resultant test solution being stabilised by adjusting the pH to ca 1 and storing it below $0^{o}C$ (125). The third problem is associated with the test itself. These pyrrole solutions are reacted with dimethylaminobenzaldehyde in the presence of acid to give highly coloured solutions (see Chapter 2) whose optical densities are then measured. However, it has been noted that these coloured solutions are unstable to varying degrees which makes the quantitative determination of pyrroles extremely difficult and, even more difficult, is the comparison of such results obtained from different laboratories.

Before continuing the discussion of these practical problems a brief account of the role played by these two molecules

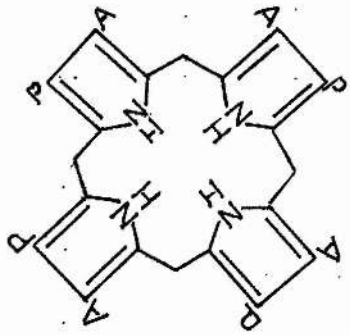
Glysine + Succinyl CoA $\xrightarrow{\text{ALA synthetase}}$ $H_2NCH_2CO(CH_2)_2CO_2H$ (ALA) $\xrightarrow{\text{ALA dehydrase}}$



porphobilinogen

XXXIV

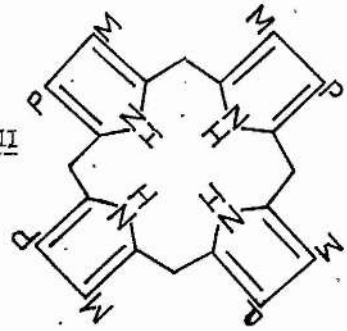
$\xrightarrow[\text{PBG isomerase}]{\text{PBG deaminase}}$



Uroporphyrinogen III

LX

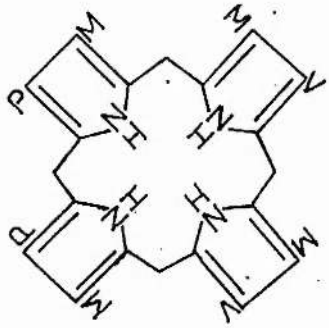
Uroporphyrinogen decarboxylase



Coproporphyrinogen III

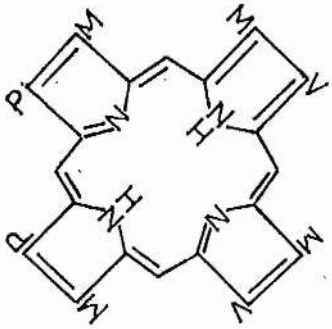
LXI

$\xleftarrow{\text{Coproporphyrinogen III oxidase}}$



Protoporphyrinogen IX

LXII



Protoporphyrin IX

LXIII

$\xrightarrow[Fe^{++}]{\text{Ferrochetalase}}$ HEME

A = Acetic acid
P = Propionic acid
V = Vinyl
M = Methyl

SCHEME 12

in biological and clinical chemistry will be given. Porphobilinogen is a precursor in the synthesis of heme (Scheme 12). Initially δ-aminolevulinic acid (ALA) is formed by condensation of succinyl-CoA and glycine in the presence of pyridoxal phosphate. Two molecules of ALA are condensed to form one of porphobilinogen which is, in turn, self-condensed to give uroporphyrinogen III, which contains four porphobilinogen moieties connected by methylene bridges. Through a combination of decarboxylation and oxidation, uroporphyrinogen III is converted into coproporphyrinogen III, protoporphyrinogen IX and finally protoporphyrin IX, the immediate precursor of heme. As noted in this sequence the true intermediates in the biosynthesis of heme are the colourless porphyrinogens. These are unstable and are easily oxidised to the corresponding porphyrin pigments which are no longer in the metabolic scheme but are found as excretion products. The determination of these porphyrins, porphobilinogen and ALA in biological samples is the basis for the detection of various metabolic abnormalities. Two recent reviews (126, 127) have been written on this subject.

Porphobilinogen is found in the urine of most people; the normal daily output is up to 2.0 mg (128). However, many patients suffering from the painful and delirious metabolic disease porphyria excrete much greater quantities of this compound (10-200 mg per day (127)), and the detection of such quantities is used as a confirmatory test for this disease. The urine is, also, often dark red in colour due to the presence of a

large quantity of associated porphyrin pigments. There are various different types of porphyrias and a classification adapted from that of Levere and Kappas (129) is given in Table 18.

Table 18

Classification of the porphyrias

1. Erythropoietic
   a) Congenital erythropoietic porphyria (CEP)
   b) Congenital erythropoetic protoporphyria (EPP)

2. Hepatic
   a) Hereditary
      (1) Acute intermittent porphyria (AIP)
      (2) Congenital cutaneous hepatic porphyria (congenital CHP)
   b) Acquired cutaneous hepatic porphyria (acquired CHP)
      - toxic or drug-induced porphyrias

Only two of these porphyrias, however, lead to a substantial increase in the urinary porphobilinogen content, namely, acute intermittent porphyria (AIP) and congenital cutaneous hepatic porphyria (congenital CHP).

Acute intermittent porphyria is the hepatic porphyria most commonly found in northern Europe and North America. Acute abdominal pain, sometimes with constipation and vomiting, is the typical symptom. It is inherited as a dominant characteristic. The overproduction of ALA synthetase in the liver causes large amounts of ALA and porphobilinogen to be produced and excreted in the urine. A deficiency of PGB deaminase and PBG isomerase rules out any increase in porphyrin excretion through the usual metabolic pathway, although nonenzymic polymerisation and

oxidation converts some of the excreted porphobilinogen to the coloured pigments uroporphyrin and porphobilin. Thus, the urine may darken on standing.

Hereditary cutaneous hepatic porphyria is in some ways similar to acute intermittent porphyria, especially in its acute phase. ALA and porphobilinogen are excreted in abnormal amounts in the urine due to the increased hepatic production of ALA synthetase. However, since there is no deficiency of the enzymes PGB deaminase and PBG isomerase, uroporphyrin III, coproporphyrin III and protoporphyrin are also formed in relatively large quantities and can also be found in the urine. The disease is inherited as a dominant characteristic.

Historical note.- King George III, often called 'Mad King George' due to his, at times, irrational behaviour, is now known to have suffered from porphyria. Although the porphyrias have only recently been recognised, the medical records kept by his physicians indicate clearly that the king suffered from such a metabolic disease; indeed his urine was often described as being the colour of port wine. His 'madness' is now thought to have been a consequence of both the extreme pain he suffered and the wrong treatment prescribed by his physicians who believed him to be psychotic. Two recent researchers in this field, Macalpine and Hunter (130) have traced signs of porphyria in his family as far back as his 16th-century ancestor, Mary, Queen of Scots. From her descendants the disorder spread to both the Hanoverian

and Prussian royal lines. Those afflicted by the disease include James VI and I of Scotland, Queen Anne, George IV, Edward, the Duke of Kent (Queen Victoria's father), Frederick William I and Frederick the Great.

In contrast to the detailed knowledge available for the role of porphobilinogen in metabolic chemistry, the data concerning cryptopyrrole are scant. It is not clear what part cryptopyrrole plays in the metabolic mechanism nor, in fact, if it possesses such a role at all. In 1961, Irvine (131) reported a substance, which he called the 'mauve factor' because of its positive Ehrlich reaction, to be present in statistically significant amounts in the urine of patients suffering from psychosis. It has subsequently been reported by several investigators, (132, 133, 134) that the factor is usually present in 30-60% of psychotic patients. Indeed, it has been suggested by Hoffer (135) that the production of this mauve colour in the Ehrlich reaction is diagnostic of a disease entity (malvaria). The identification of this compound, hampered by its extreme sensitivity to mild procedures, has been carried out using ATC (autotransfer chromatography) and was found to be identical to cryptopyrrole (136). The chromatograms of cryptopyrrole and the 'mauve factor' are identical, characteristic, and in the case where water is used as the solvent, complex in that they show five distinct spots. Under elution with water the five components of these chromatograms revert to one component which has been identified as dicryptopyrrole ether by means of high resolution mass spectrometry (136, 137). Recently, it has

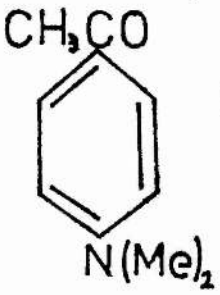
been shown that this multiple spot formation is partly an artifact of chromatography (138).

Confirmation of the 'mauve factor' as cryptopyrrole has been made by Sohler et al (139) using paper and thin-layer chromatography.

Although it is not clear whether or not cryptopyrrole is a cause of pychosis or whether it is a product formed due to some metabolic failure within the patient, Sohler (139) have eliminated it as being a possible stimulant metabolite causing over-arousal in the schizophrenic patient.

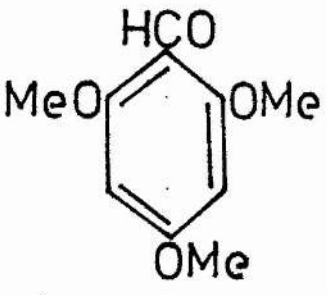
Recently, a rapid screening procedure has been proposed (140), using the Ehrlich test on urine, to detect cryptopyrrole and other urinary pyrroles to distinguish a schizophrenic sub-population.

The stability of cryptopyrrole in water and its detection and quantitative determination are considered on subsequent pages.



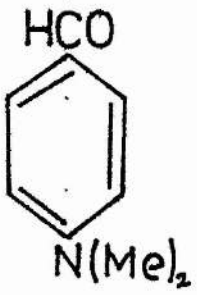
DMAA

LXIV



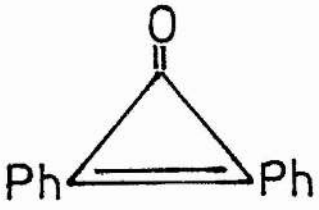
TMB

LXV



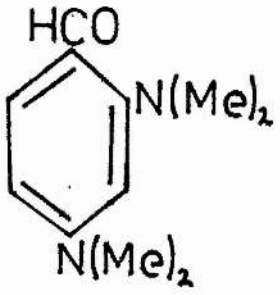
DMAB

XXIX



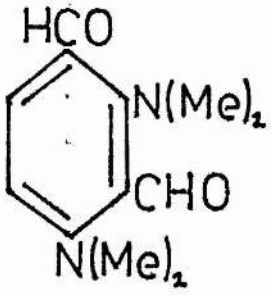
Diphenylcyclopropenone

LXVI



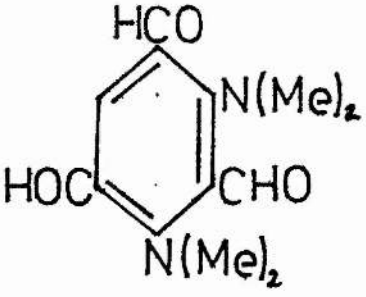
BDMAB

LXVII



BDMAI

LXVIII



BDADFB

LXIX

FIGURE 10 Reagents used in the determination of cryptopyrrole and porphobilinogen

Results and discussion.-

The present study can be separated into three sections. Firstly, a series of reagents, which are in some ways similar to Ehrlich's aldehyde (DMAB), were either obtained commercially or synthesised in the laboratory. Secondly, an attempt was made to determine whether any of these compounds gave a stable coloured solution when reacted with various pyrrole test solutions. Lastly, the conditions under which these pyrrole test solutions were most stable were investigated. The sources of these reagents are discussed in the experimental section and the results of their coupling reactions with pyrroles and the stability of these pyrrole solutions are outlined below. The reagents used in this study are shown in Figure 10.

(1) 4-Dimethylaminoacetophenone (DMAA)

A 1 M DMAA solution was prepared by dissolving DMAA (0.81 g) in 3 M HCl (5 ml).

Reaction with pyrrole.- The above solution (1 ml) was added to an aqueous pyrrole solution (1 ml) and the spectral changes observed. The spectrum showed an absorption at 490 nm which took ca 30 minutes to reach a maximum and which was subsequently stable for at least 1 hour. Increasing the concentration of DMAA by a factor of two increased the rate at which the absorption maximum was reached (ca 20 minutes) but since this solution was almost saturated no further increases in DMAA concentration were made. Increasing the acid concentration to

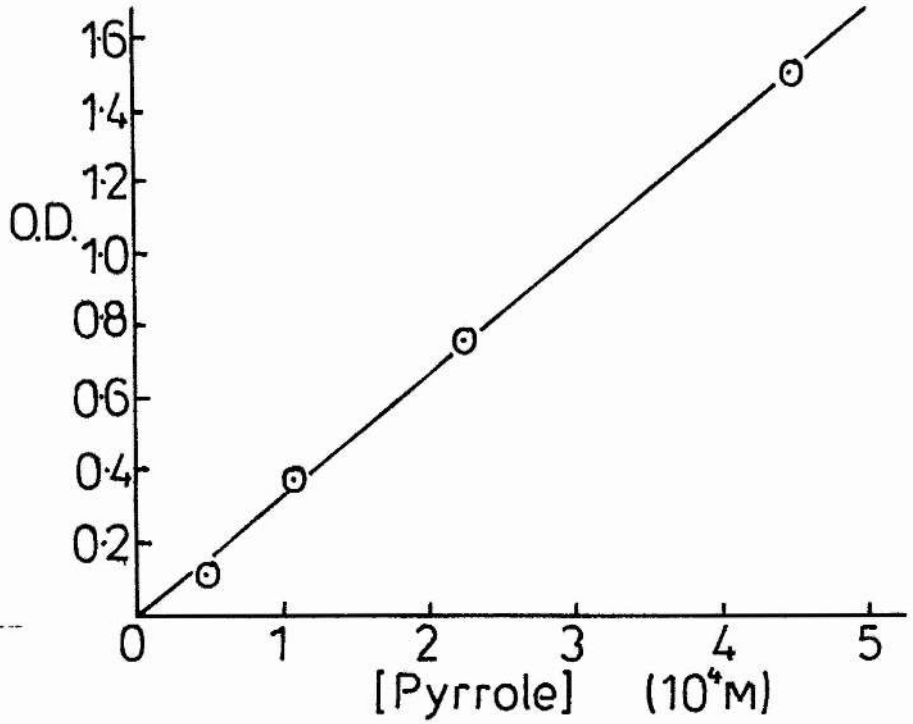


FIGURE 11 Variation of optical density at 490nm with pyrrole concentration after reaction with DMAA

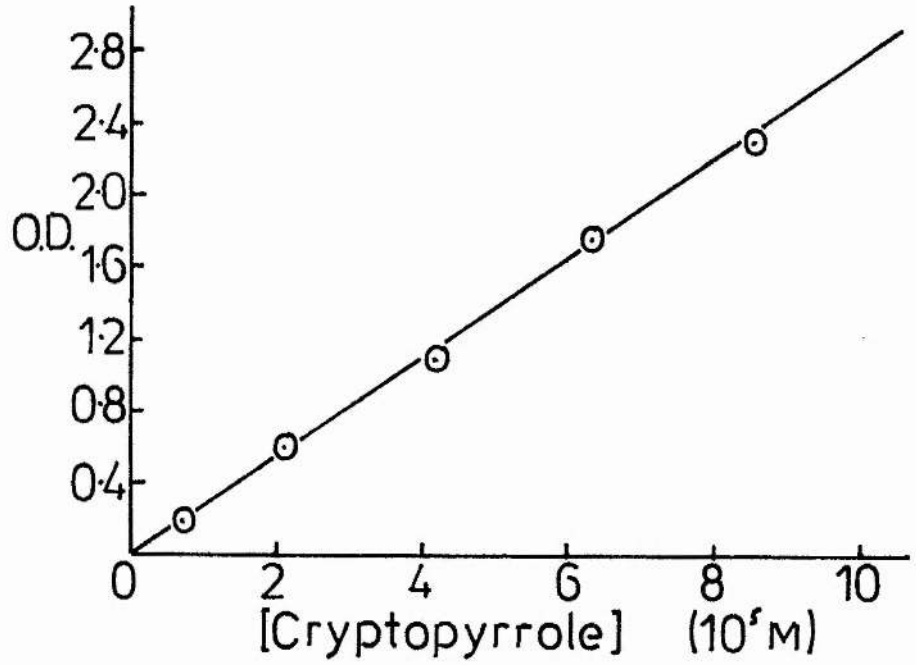


FIGURE 12 Variation of optical density at 486nm with cryptopyrrole concentration after reaction with DMAA

7 M had little effect on the rate of reaction, but did move the absorption maximum to a longer wavelength (505 nm). With the standard 1 M DMAA solution above the absorption maxima were found to show a linear dependence on the pyrrole concentration (Figure 11).

Reaction with cryptopyrrole.- The standard 1M DMAA solution (1 ml) was added to a cryptopyrrole solution (1 ml) made up in 0.1 M HCl. The resulting spectrum showed an absorption at 486 nm which took 15 minutes to reach a maximum and which was stable for at least 1 hour. The absorption maxima recorded for several cryptopyrrole solutions showed a linear dependence on the pyrrole concentration (Figure 12).

Reaction with porphobilinogen.- The 1M DMAA solution (1 ml) was added to a porphobilinogen solution (1 ml) made up in 0.1 M HCl and the spectrum observed. After 45 minutes no reaction had taken place.

(2) 2,4,6-Trimethoxybenzaldehyde (TMB)

A 0.01 M TMB solution was prepared by dissolving TMB (0.1 g) in ethanol (50 ml). In the following tests this solution (1 ml) was added to 3N HCl (1 ml) and to the resulting mixture was added the appropriate pyrrole solution (1 ml).

Reaction with cryptopyrrole.- The above test was carried out using various cryptopyrrole solutions prepared in 0.1 M HCl and the spectra observed. These showed an absorption

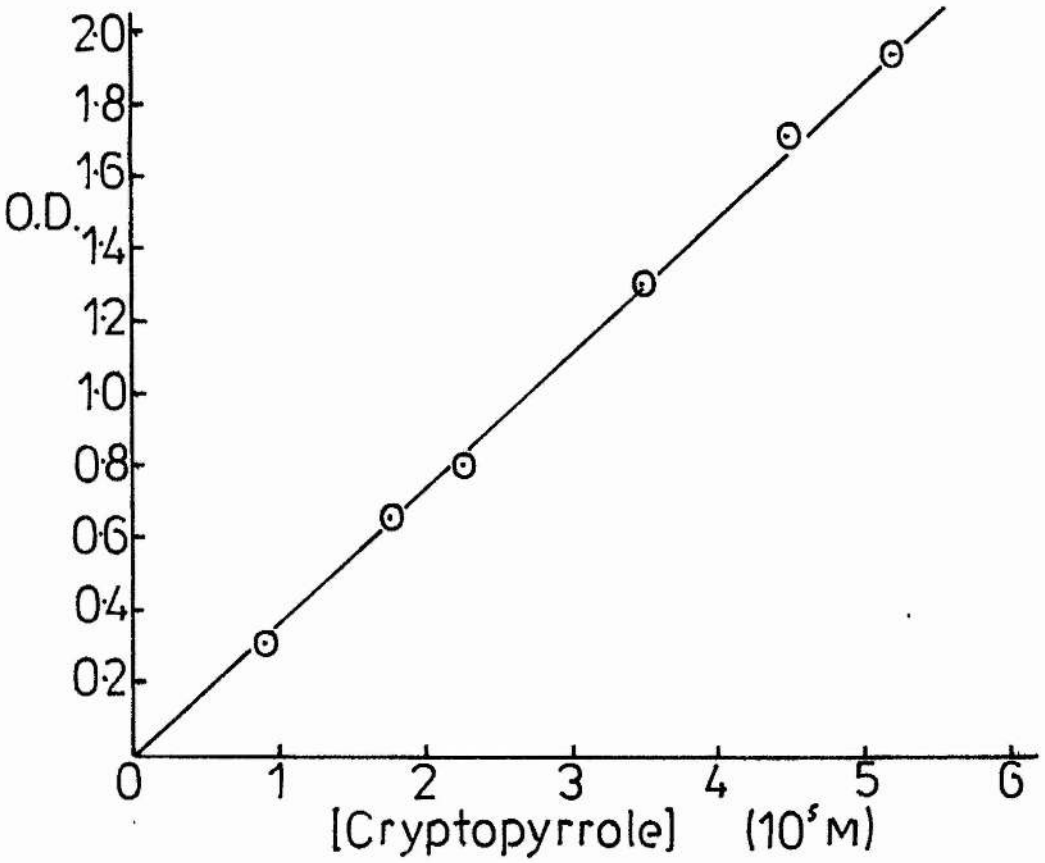


FIGURE 13 Variation of optical density at 460nm with cryptopyrrole concentration after reaction with TMB

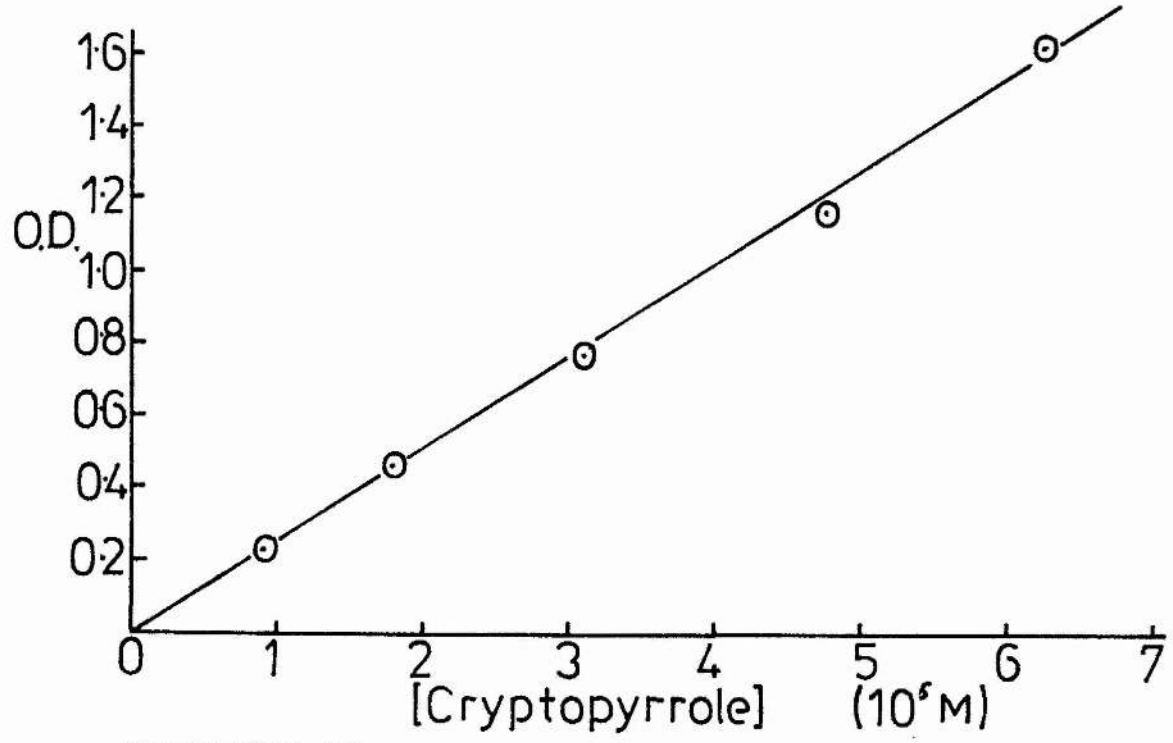


FIGURE 14 Variation of optical density at 540nm with cryptopyrrole concentration after reaction with DMAB

at 460 nm which reached a maximum after 15 minutes and which was stable for at least 4 hours. A plot of optical density versus cryptopyrrole concentration is linear and passes through zero (Figure 13).

Reaction with porphobilinogen.- The above test was carried out using a porphobilinogen solution made up in 0.1 M HCl but no reaction had taken place after 1 hour.

(3) 4-Dimethylaminobenzaldehyde (DMAB)

Three separate DMAB solutions were prepared as follows:-

(A) DMAB (1.86 g) was dissolved in 3N HCl (50 ml) to give a 0.25 M solution.

(B) DMAB (0.074 g) was dissolved in ethanol (50 ml) giving a 0.01 M solution.

(C) 'Modified' Ehrlich's Reagent (124, 141) - To DMAB (1 g) in glacial acetic acid (30 ml) was added 70% perchloric acid (8 ml) and this diluted to 50 ml with glacial acetic acid.

Tests with solution (A) above.-

Reaction with cryptopyrrole.- Solution (A) (1 ml) was added to a cryptopyrrole solution (1 ml) prepared in 0.1 M HCl and the spectrum taken. This showed an absorption at 542 nm which had reached a maximum within the first few minutes after mixing. The optical density maximum was stable for at least 1 hour.

Reaction with porphobilinogen. - Solution (A) reacts fairly rapidly with an aqueous porphobilinogen solution and this was

studied on the stopped-flow apparatus. Using a 4.8×10^{-5} M porphobilinogen solution the reaction was studied at 556 nm. The reaction showed first order kinetics and a rate constant of 2.98×10^{-2} s^{-1} was calculated. When the reaction was studied using the Unicam SP800 spectrometer, the absorbance had already reached a maximum and was beginning to decrease by the time the first spectrum could be taken (ca 2 minutes). After a further $\frac{1}{2}$ hour the absorbance had decreased to ca $\frac{3}{4}$ of the maximum value and after $1\frac{1}{2}$ hours the value was only $\frac{1}{3}$ of the maximum.

Solution (B) was used in the quantitative determination of cryptopyrrole, the test employed being similar to that with the TMB solution described above. In this case the optical densities were measured at 540 nm 25 minutes after mixing. The resulting absorption maxima were stable for several hours. A graph (Figure 14) of cryptopyrrole concentration versus optical density is linear and passes through the origin.

Solution (C), developed by Mauzerall and Granick (124) as an improved reagent for the determination of porphobilinogen, was used in several experiments in an attempt to substantiate this claim. Several porphobilinogen solutions were prepared and reacted with this reagent and the results obtained were in agreement with those of the above workers. Thus, for the series of pyrrole solutions used the absorbance reached a maximum within the time interval 5 to 15 minutes after mixing and were stable from 3 to 15 minutes. The maximum optical density was directly proportional to the porphobilinogen concentration.

Note. - DMAB and TMB were used for the detection of cryptopyrrole on TLC plates. Thus, a 1.1×10^{-4} M cryptopyrrole solution was spotted on a TLC plate and sprayed with a 0.1 M DMAB solution prepared in 3N HCl. A slight colouration appeared only after several hours. When a spot of pure cryptopyrrole was sprayed an immediate very intense red colour appeared which was stable overnight. With a 0.015 M TMB solution prepared in ethanol and mixed with an equal volume of 5M HCl no reaction was observed with the aqueous cryptopyrrole solution but with the pure cryptopyrrole a yellow colour appeared which was stable.

(4) Diphenylcyclopropenone.

A 0.023 M solution of diphenylcyclopropenone was prepared by dissolving diphenylcyclopropenone (0.20 g) in ethanol (50 ml).

An attempt was made to react the above reagent with an aqueous pyrrole solution employing the following experimental methods:-

(a) the diphenylcyclopropenone solution (1 ml) was added to an aqueous pyrrole solution (1 ml) resulting in no reaction.

(b) the above reagent (1 ml), 3N HCl (1 ml) and a pyrrole solution (1 ml) were mixed but no reaction took place.

(c) as for (b) but with concentrated HCl; again no reaction occurred.

(d) as for (b) with the resulting solution being heated on a steam bath for 15 minutes; no reaction took place.

In place of the above diphenylcyclopropenone solution, a saturated solution of the reagent was prepared in ethanol and

used as above. Under these experimental conditions no reaction took place.

An attempted coupling of diphenylcyclopropenone with pyrrole, under synthetic conditions, using the method of Lloyd et al (127) and that described in Chapter 2 in obtaining the highly coloured salts with DMAB, was carried out but resulted in a tar formation in both cases.

(5) 2,4-Bis(dimethylamino)benzaldehyde (BDMAB)

Reaction with N-methylpyrrole.- Two solutions of BDMAB were prepared ((a) a 0.008 M solution in 5M HCl, (b) a 0.015M solution in 5M HCl) and 1 ml of each solution was added to 1 ml of an aqueous N-methylpyrrole solution and the spectra observed. These showed considerable variation with time. For solution (a), 1 minute after mixing, the maximum absorption was at 570 nm but within 5 minutes this had moved to 528 nm. The absorbance then increased slightly over the next 15 minutes after which time it was stable for the next 1 hour. For solution (b) the spectrum again showed a maximum absorption at 570 nm which moved to 528 nm where it immediately began to fade.

A 0.124 M BDMAB solution was also prepared in 2M HCl and reacted with the N-methylpyrrole solution. The spectrum of the resulting solution showed an absorption at 544 nm which was at a maximum 5 minutes after mixing but immediately began to fade.

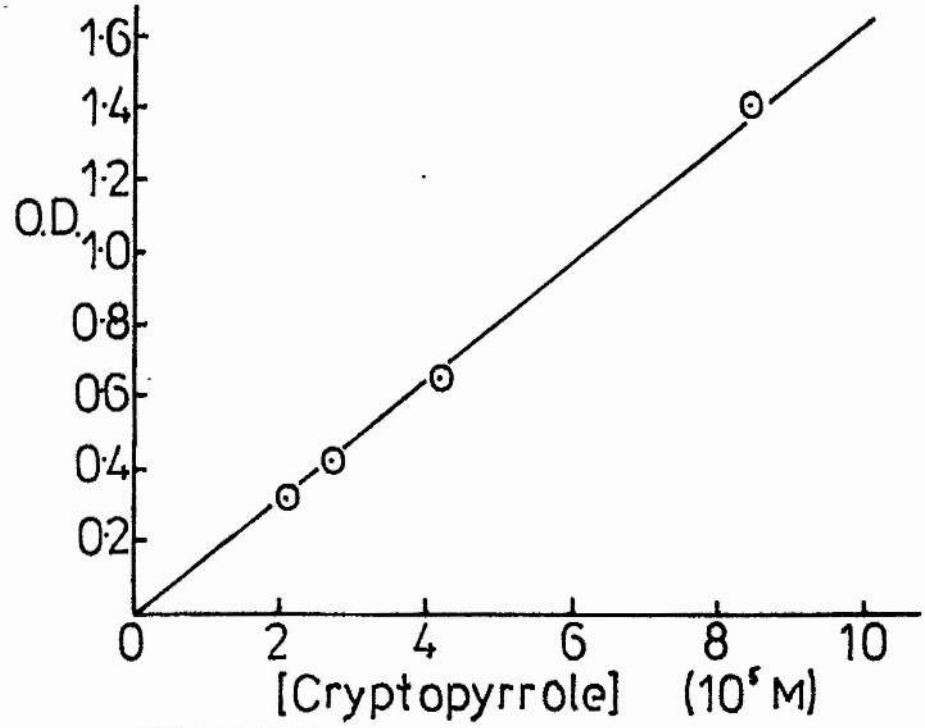


FIGURE 15 Variation of optical density at 532nm with cryptopyrrole concentration after reaction with BDMAB

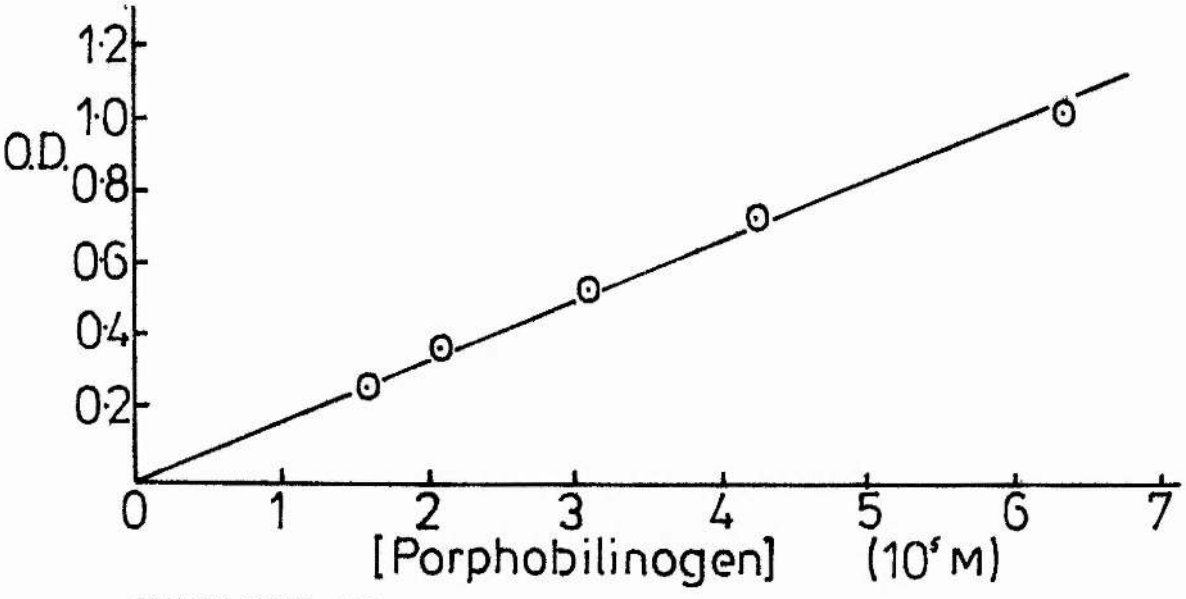


FIGURE 16 Variation of optical density at 534nm with porphobilinogen concentration after reaction with BDMAB

Reaction with cryptopyrrole.- A 0.15 M BDMAB solution was prepared by dissolving BDMAB (0.24 g) in 2M HCl (10 ml). This solution (1 ml) was added to a cryptopyrrole solution (1 ml) prepared in 0.1M HCl as before. The resulting solution showed an absorption at 532 nm which reached a maximum value after 15 minutes and which was stable thereafter for at least 2 hours. A linear dependence between optical density and the cryptopyrrole concentration was found (Figure 15).

Reaction with porphobilinogen.- The reaction between BDMAB and a porphobilinogen solution prepared in 0.1 M HCl was carried out as described before using a variety of different BDMAB solutions. In all cases the spectra showed a maximum absorption at 534 nm. The results are given in Table 19.

Since the result found for solution (iii) was obviously the most promising , a quantitative test for the determination of porphobilinogen was devised based on this BDMAB solution composition.

A porphobilinogen solution was prepared in 0.1M HCl and a 1 ml sample of this was reacted with 1 ml of 'modified' Ehrlich's reagent. The maximum optical density was measured and from this the concentration of the porphobilinogen solution was calculated to be 0.0285 g/l (compared to 0.0320 g which was weighed out) using the formula given by Mauzerall and Grunick (124).

Table 19

Results for the reaction between BDMAB and porphobilinogen

Composition of BDMAB solution	Result
(i) 0.13 M solution of BDMAB in 5M HCl	absorption reached a maximum value after 15 minutes at room temperature and then began to fade
(ii) 0.10 M solution of BDMAB in 0.5M HCl	absorption had not reached a maximum value $2\frac{1}{2}$ hours after mixing
(iii) 0.13 M solution of BDMAB in 2M HCl	the resulting solution was heated in a water bath at 50°C for 15 minutes. 20 minutes after mixing the absorption had reached a maximum value and was stable for 40 minutes
(iv) 0.14 M solution of BDMAB in 1M HCl	the resulting solution was heated in a water bath at 50°C for 25 minutes. 30 minutes after this water bath treatment the absorption was still increasing
(v) an attempt was made to prepare a solution of BDMAB in a perchloric/glacial acetic acid mixture but when the perchloric acid was added a reaction occurred and a white precipitate was formed	
(vi) 0.13 M solution of BDMAB in glacial acetic acid	no reaction took place

The test using BDMAB consisted of adding 1 ml of the porphobilinogen solution to 1 ml of the standard BDMAB reagent (solution (iii)) in a 5 ml stoppered volumetric flask. This was then placed in a water bath at 50°C for 15 minutes, the flask shaken and the contents poured into a cuvette. This was then

allowed to stand at room temperature for 5 minutes before the optical density was measured. In all but one case tested the optical density had reached a maximum by this time and in this one outstanding case a maximum was achieved within a further 5 minutes. The stability of these maxima varied from 10 minutes up to 40 minutes. A linear dependence between the maximum optical density and the porphobilinogen concentration was found (Figure 16).

(6) 2,4-Bis (dimethylamino)isophthalaldehyde (BDMAI)

A 0.84×10^{-2}M solution of BDMAI was prepared in 5M HCl and reacted with an aqueous N-methylpyrrole solution as before. The spectrum showed an absorption at 570 nm which had reached a maximum 1 minute after mixing but very rapidly began to fade.

When a 4.5×10^{-2} M solution of the reagent, prepared in 1M HCl, was added to an aqueous porphobilinogen solution no reaction took place within the first 3 hours after mixing.

(7) 2,4-Bis(dimethylamino)-3,5-diformylbenzaldehyde (BDADFB)

A 0.65×10^{-2} M solution of BDADFB was prepared in 2.5 M HCl and reacted with an aqueous N-methylpyrrole solution and the spectrum recorded. This showed an absorption at 505 nm which had not reached a maximum value two hours after mixing. Increasing both the BDADFB and the acid concentrations increased the rate of reaction.

This reagent did not react with an aqueous porphobilinogen solution.

The stability of various aqueous pyrrole solutions.-

Dilute solutions (ca 10^{-5}M) of N-methyl-, 2,3-dimethyl-, 1,2,3-trimethyl-, 1,2,5-trimethyl- and 2,3,5-trimethylpyrrole, were made up in distilled water and their stability as a function of time was monitored by reacting 1 ml aliquots of the solutions with TMB and DMAB reagents at various time intervals. For N-methyl-, 2,3-dimethyl- and 1,2,3-trimethylpyrrole, these solutions were stable for at least 12 hours. However, for the 1,2,5-trimethyl- and 2,3,5-trimethylpyrrole solutions slow decomposition occurred over several hours. In both cases the pyrrole concentration had fallen to ca $\frac{1}{2}$ of the original value after 6 hours.

It has been reported that porphobilinogen undergoes decomposition in urine when left standing; after 10 hours at 20-37°C as much as 70% may disappear (142). Cookson and Rimington (143) have reported that all porphobilinogen in a urine sample having an alkaline pH had disappeared in 17 days whereas in 0.5 M HCl 55% had disappeared in the same time interval. At least 2 weeks stability has been found (125) by adjusting the pH of the urine to 6-7 and storing it below 4°C. A solution of porphobilinogen in 0.1 M HCl was prepared and stored below 5°C for 10 days. Aliquots were taken and tested with both 'modified' Ehrlich reagent and a BDMAB solution at 24 hour intervals. Less than 5% decomposition had occured in this period of time.

Several dilute solutions (ca 10^{-5}M) of cryptopyrrole were prepared in distilled water and their stability monitored as before.

It was found that they were unstable. Thus, an aqueous solution of cryptopyrrole (4×10^{-5}M) kept at 25°C showed a first order decrease in cryptopyrrole concentration with a rate constant of $8.8 \times 10^{-5} s^{-1}$ and a half-life of 2.7 hours. An attempt was made to identify the product(s) of this decomposition by refluxing a sample of cryptopyrrole with water for 24 hours. A light brown powder was obtained which gave no mass spectrum and no positive test with DMAB. It was most probably a polymer. Irvine et al (137) have detected dicryptopyrryl ether as one product formed during chromatography but the complexity of the chromatograms would tend to indicate that this is not the only product formed under these conditions.

However, the u.v. spectrum of cryptopyrrole in 0.1M HCl (λ_{max} 262 nm) remains unchanged for many hours and clearly protonation renders the compound not susceptible to hydrolysis. This has been confirmed by monitoring the stability of these solutions with a TMB reagent. Although Fischer (144) has reported that cryptopyrrole polymerises in acid, this has not been confirmed by later workers (22, 25) and it is now thought to form the simple hydrochloride salt in HCl.

Therefore, based on the information given above, it would appear that the more substituted the pyrrole the less stable are its aqueous solutions. Protonation of these pyrroles (at least in the cases of cryptopyrrole and porphobilinogen) by dissolving them in relatively weak acid renders them resistant to hydrolytic attack.

Conclusions.-

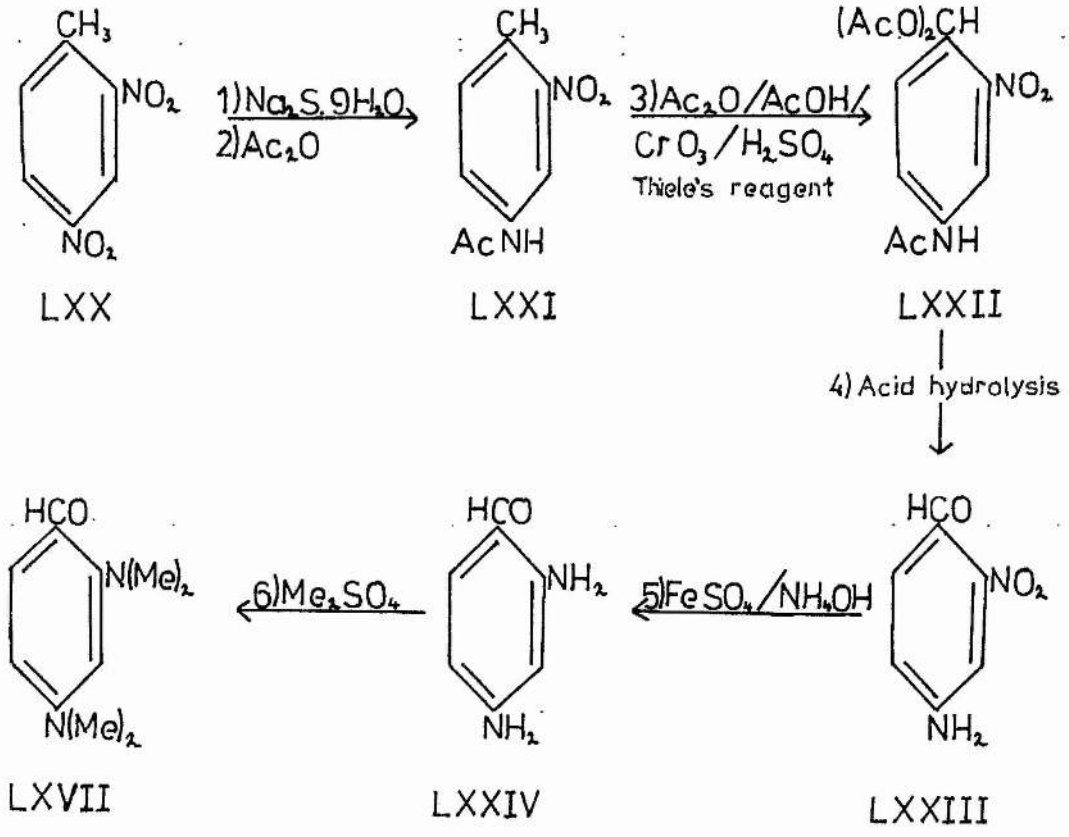
The problems involved in the clinical determination of both porphobilinogen and cryptopyrrole have been outlined above. Separation of these compounds from other components in biological fluids can be effected using chromatography. It has been shown by Haeger-Aronson (155) that recovery of porphobilinogen from urine using column chromatography was greater than 96%. It should be noted, however, that extensive chromatography of aqueous cryptopyrrole solution should be avoided due to the hydrolytic lability of this compound under these conditions.

The quantitative determination of cryptopyrrole is relatively straightforward. Once the test solution has been made acidic it is stable for a considerable time. The actual test can be carried out using either DMAB, TMB or BDMAB, all of which give stable coloured solutions whose optical density is directly proportional to the cryptopyrrole concentration. At the pH of urine ($5 \longrightarrow 8.5$), however, cryptopyrrole is found to be unstable. Thus, a substantial amount of cryptopyrrole may well be lost before the test can be carried out. This would mean that the amount of cryptopyrrole calculated to be in any urine sample may only be a fraction of the total cryptopyrrole produced by the patient.

In contrast to that of cryptopyrrole, the determination of porphobilinogen is far from easy. The isolation of porphobilinogen is straightforward and the test solution can be made

stable by adjusting its pH to ca 1 and storing it at 0°C. However, no completely satisfactory reagent has been developed for its quantitative determination. Using DMAB dissolved in hydrochloric acid the reaction is fast and the colour obtained is very unstable. 'Modified' Ehrlich's reagent has the advantages that the reaction is slower and, therefore, more easily monitored, and the colour obtained has a certain degree of stability (ranging from 3 to 15 minutes depending on the sample). The disadvantages of this reagent are that the time taken for the absorbance to reach a maximum varies from sample to sample, the time the maximum absorbance is stable varies from a few minutes up to at least 15 minutes and the reagent itself is unstable over 24 hours. The main advantage of the method developed by Mauzerall and Granick (124), incorporating this reagent, is that it has been used successfully under 'experimental' conditions.

The apparent advantage in using BDMAB is the increased stability of the colour formed, although, as with the above reagent, the length of time the colour is stable varies (from 10 up to 40 minutes). Disadvantages of this reagent include the necessity for heating the resulting solution in a water bath and the fact that it has not been used in anything other than 'ideal' test conditions. The BDMAB reagent is more stable than the 'modified' Ehrlich reagent and was found to be stable for at least a week when kept refrigerated.


CH3
NO2
NO2
LXX
1) Na2S.9H2O
2) Ac2O
CH3
NO2
AcNH
LXXI
3) Ac2O/AcOH/
CrO3/H2SO4
Thiele's reagent
(AcO)2CH
NO2
AcNH
LXXII
4) Acid hydrolysis
HCO
NO2
NH2
LXXIII
5) FeSO4/NH4OH
HCO
NH2
NH2
LXXIV
6) Me2SO4
HCO
N(Me)2
N(Me)2
LXVII

SCHEME 13

Experimental.-

Materials.- The hydrochloric acid used was AnalaR grade. The 4-dimethylaminobenzaldehyde was purchased and was AnalaR grade. Trimethoxybenzaldehyde was purchased from Aldrich Chemical Co. and recrystallised three times from ethanol before use.

4-Dimethylaminoacetophenone was prepared by methylation of 4-aminoacetophenone using the method Suzuki and Nagawa (145). Diphenylcyclopropenone was bought from Koch-Light and used without further purification.

A sample of 2,4-bis(dimethylamino)benzaldehyde was prepared by the route outlined in Scheme 13. 2,4-Dinitrotoluene was first converted to 2-nitro-4-acetylaminotoluene by the method of Hillers et al (146). The amide was then converted to 2,4-diaminobenzaldehyde by the method of Ricci (147) and finally N-methylated using dimethylsulphate to give the required compound. At stage 3) (Scheme 13) no solid precipitate was obtained on neutralisation as found by Ricci (147) but this neutralised reaction mixture on extraction with methylene chloride yielded a sticky red liquid which was dissolved in a minimum amount of boiling methylene chloride and refrigerated. After 24 hours a yellow-white solid was precipitated. This proved to be the required compound and was subsequently recrystallised from aqueous methanol.

Stage 6) (Scheme 13) was carried out as follows:-

2,4-diaminobenzaldehyde (1.36 g) was stirred under reflux with a solution of anhydrous sodium carbonate (30 g) in water (80 ml).

To this was added dimethyl sulphate (30 g) dropwise over 45 minutes. The solution was then refluxed a further 15 minutes, allowed to cool and extracted with chloroform (2 x 30 ml). The chloroform extracts were washed, dried and evaporated to give the desired product (1.35 g, 70%). This can be purified by distillation (B.P_{20}=190°C) or by chromatography similar to that described below.

Reaction between 1,3-bis(dimethylamino)benzene and a phosphoryl chloride/dimethylformamide solution.-

1,3-Bis(dimethylamino)benzene was prepared from 1,3-diaminobenzene by the method of Torf and Khromov-Borisov (148). This was reacted with phosphoryl chloride/dimethylformamide under two sets of experimental conditions leading to the three products, BDMAB, BDMAI and BDADFB.

(a) Phosphoryl chloride (26 g) was added dropwise and with stirring to dimethylformamide (22 g) below 15°C. To this stirred solution was added 1,3-bis(dimethylamino)benzene (8 g) dropwise with the temperature being kept below 25°C. The resultant solution was then stirred for 2 hours at 65-70°C, cooled and poured onto ice/water (500 ml). This was basidified (pH 10 $\longrightarrow$ 11) by adding sodium hydroxide solution (30 g NaOH in 70 ml of water) dropwise with vigorous stirring. The mixture was heated on a steam bath for 10 minutes, cooled and extracted with chloroform (3 x 100 ml). The organic extracts were washed with water (2 x 50 ml), dried over $CaCl_2$ and evaporated to give

2,4-bis(dimethylamino)-3, 5-diformylbenzaldehyde (BDADFB). This was recrystallised from a hexane/ethyl acetate mixture to yield yellow needles (m.p. = 117-119°C, m/e = 248). The n.m.r. spectrum, taken in $CDCl_3$, showed the following peaks (integral values in brackets) relative to TMS - 3.3δ (12), 8.1δ (1), 9.3δ (1), 9.75δ (2).

Analysis - calculated 62.84% C, 6.40% H, 11.28 % N

found 62.57% C, 6.58 % H, 11.14 % N.

(b) Phosphoryl chloride (7.65 g) was added dropwise with good stirring to a mixture of 1,3-bis(dimethylamino)benzene (8.2 g) and dimethylformamide (14.6 g) with the temperature being kept below 10°C. The temperature of the stirred mixture was allowed to rise to room temperature over 2 hours and stirred a further $\frac{1}{2}$ hour. It was then added to ice/water (400 ml) and basidified (pH 10 $\longrightarrow$ 11) by adding sodium hydroxide solution (10 g NaOH in 25 ml of water) dropwise and with vigorous stirring. The resulting solution was heated on a steam bath for 10 minutes, cooled and extracted with chloroform (3 x 75 ml). The organic extracts were washed, dried over $CaCl_2$ and evaporated to give a sticky liquid. This was dissolved in a little chloroform and absorbed onto a 1 metre alumina chromatography column prepared in petroleum. Elution with petroleum spirit/ether (50:50) gave first unreacted starting material and then 2,4-bis-(dimethylamino)benzaldehyde (BDMAB) as a yellow band. A yellow band of 2,4-bis(dimethylamino)isophthalaldehyde (BDMAI) still remained on the column and this could be removed physically

or by elution with a more polar solvent.

2,4-bis(dimethylamino)benzaldehyde.- yield = 3.3 g (35%), m/e=192

The n.m.r. spectrum, taken in $CDCl_3$, showed the following peaks (integral values in brackets) relative to TMS - 2.85 δ (6), 3.00 δ (6), 6.05 δ doublet (1), 6.25 δ quartet (1), 7.60 δ doublet (1) and 9.9 δ (1).

Analysis - calculated 68.72% C, 8.39 % H, 14.67 % N

found 68.93% C, 8.33 % H, 14.73 % N

Note.- BDMAB is best stored at 0°C since it was found to undergo slow decomposition at room temperature. Before preparing the standard BDMAB solutions used in the pyrrole tests the BDMAB can be distilled or more conveniently dissolved in boiling petroleum spirit (40-60°C), this solution being filtered hot. It was found that the impurity produced in decomposition was insoluble in this solvent. Evaporation of the petroleum gives the BDMAB which can be used in the tests.

2,4-Bis(dimethylamino)isophthalaldehyde.- yield = 2.5 g(25%), m/e = 220, m.p. = 123-125°C (from hexane-ethyl acetate mixture).

The n.m.r. spectrum, taken in $CDCl_3$, showed the following peaks (integral values in brackets) relative to TMS - 3.15 δ (6), 3.30 δ (6), 6.45 δ doublet (1), 7.70 δ doublet (1), 9.50 δ (1) and 9.80 δ (1).

Analysis - calculated 65.43 % C, 7.32 % H, 12.71 % N

found 65.01 % C, 7.08 % H, 12.42 % N

Attempted synthesis of 2,4-bis(dimethylamino)acetophenone (BDMAA).-

1,3-Bis(dimethylamino)benzene was prepared by the method reported previously (148) and various attempts at acylation of this compound were made:-

(a) acylation was attempted using the method of Bourne et al (149) with the following quantities : 1,3-bis(dimethylamino)benzene (1.64 g), acetic acid (0.9 g) and trifluoroacetic anhydride (4.2 g). The mass spectrum and the n.m.r. spectrum of the resulting product indicated that this was essentially all unreacted starting material.

(b) the above experiment was carried out in an ice/water bath but again no reaction took place.

(c) the above reaction mixture was heated in a water bath at 55-60^{o}C for 9 hours. The mass spectrum and n.m.r. spectrum of the product showed it to be a mixture of the trifluoroacetyl compound and starting material.

(d) under the experimental conditions in (c) but with only 0.5 g of trifluoroacetic anhydride the only products were a reduced yield of the trifluoroacetyl compound and starting material.

(e) with acetic anhydride in place of trifluoroacetic anhydride and with the reaction mixture being heated in a water bath at 70-80^{o}C for 40 hours, only a trace amount of the required product was evident in the mass spectrum of the resulting product mixture.

(f) 1,3-bis(dimethylamino)benzene (0.84 g) was added to a mixture of acetic anhydride (1.02 g) and zinc chloride (2.75 g) and the whole refluxed for 6 hours. Isolation of the reaction product as in (a) was carried out but the mass spectrum indicated that only a trace amount of the required compound was present.

(g) as for (f) but with refluxing for 30 hours resulted in only a small quantity of product being formed.

(h) 1,3-bis(dimethylamino)benzene (0.84 g) was added to a mixture of acetic anhydride (1.02 g) and aluminium chloride (2.7 g) in 15 ml of carbon tetrachloride and refluxed for 6 hours. Only unreacted starting material was found.

(i) as for (h) but with nitrobenzene as solvent, the reaction mixture being heated at 100°C for 6 hours. However, only a trace amount of the required product was found.

An attempt to make the above compound (BDMAA) was made by first nitrating 4-dimethylaminoacetophenone (DMAA) with subsequent reduction, and N-methylation of any nitro groups introduced. However, using the experimental conditions described below the initial nitration failed.

(a) DMAA (2.5 g) was added very slowly, with stirring, to fuming nitric acid (6 ml) at 0°C and the mixture stirred for 1 hour at 10°C. This was then added to ice/water and a yellow precipitate was collected. This was found to be unreacted starting material. The remaining aqueous solution was extracted with chloroform but again no nitro-containing compound was found in these extracts.

(b) as for (a) but with the reaction mixture being stirred for 5 hours at 70-75°C. Again no nitro-containing compound was found.

(c) DMAA (2.5 g) was dissolved in concentrated sulphuric acid (4 ml) giving a yellow solution. This was added dropwise, with stirring, to a mixture of fuming nitric acid (4 ml) and concentrated sulphuric acid (2 ml) at 0°C. The resulting solution was stirred for 1 hour at 10°C and then poured onto ice/water. The resulting precipitate was found to be starting material.

(d) as for (c) but with the reaction mixture being stirred for 7 hours at 70-75°C. Again only starting material was found.

(e) as for (c) and (d) but with DMAA (2.5 g) being dissolved in glacial acetic acid (4 ml) and this being added to a mixture of fuming nitric acid (3 ml) and concentrated sulphuric acid (3 ml). The required product was not obtained in either case.

A further attempt to prepare BDMAA was made by attempting to couple the acid chloride of 2-nitro-4-aminobenzoic acid with ethoxymagnesiummalonic ester to give 2-nitro-4-aminoacetophenone with the intention of reducing the nitro group and finally methylating the resulting amino group.

2-Nitro-4-aminobenzoic acid.- 2-nitro-4-acetylaminotoluene (10 g) was added to a solution of potassium permanganate (20 g) in water (750 ml) and refluxed for 6 hours. The mixture was filtered hot, allowed to cool and refiltered to remove any unreacted starting material. The filtrate was then concentrated to about 100 ml ad acidified carefully with concentrated hydrochloric acid

to precipitate the product. This was recrystallised from water with a little added ethanol to give yellow-orange needles (7 g, 72%), m.p. 235-236°C (lit. 239°C (150)).

The coupling of the acid chloride of the above acid and ethoxymagnesiummalonic ester was attempted using the method of Atkinson and Biddle (151) in which the ester is generated in the reaction mixture. When this failed, however, it was decided to make the ester separately and react this with the acid chloride in dioxane which is the more common method for carrying out these couplings. The ester was prepared as a white solid using a literature method (152) but again when this was reacted with the acid chloride the synthesis failed. The most probable cause of this failure was the high reactivity of the acid chloride which reconverted to the acid very quickly on coming into contact with air.

Finally an attempt was made to synthesise 2,4,6-trinitro-acetophenone with the intention of reducing the nitro groups and methylating the resulting amino groups. 2,4,6-Trinitroethylbenzene was prepared using a literature method (153) but when the oxidation of this was attempted using the method of Adolph et al (154) only unreacted starting material was obtained from the reaction mixture.

Appendix 1

Acid-base catalysis

Acids and bases catalyse many reactions in which they are not consumed. If the rate of disappearance of a substance S (called the substrate of the catalytic reaction) is first order in S; $-d[S]/dt = k_{obs}[S]$. The first order rate constant k_{obs} for the reaction in a buffer solution may be a linear function of $[H^+]$, $[OH^-]$, $[HA]$ and $[A^-]$, where HA is the weak acid in the buffer and A^- is the corresponding anion.

$$k_{obs} = k_o + k_{H^+}[H^+] + k_{OH^-}[OH^-] + k_{HA}[HA] + k_{A^-}[A^-] \qquad (1)$$

In this equation k_o is the first order rate constant for the uncatalysed reaction and k_{H^+}, k_{OH^-}, k_{HA} and k_{A^-} are the so-called catalytic coefficients. If only the term $k_{H^+}[H^+]$ is important the reaction is said to be specifically hydrogen-ion catalysed whereas if both this term and the term $k_{HA}[HA]$ are important the reaction is said to be subject to general acid catalysis. Similarly if the terms $k_{OH^-}[OH]$ and $k_{A^-}[A^-]$ are important the reaction is said to be subject to general base catalysis.

In acid solution it is assumed that $[OH^-]$ and $[A^-]$ are very small and therefore the terms $k_{OH^-}[OH^-]$ and $k_{A^-}[A^-]$ in equation (1) can generally be ignored and the resulting equation is that for general acid catalysis:

$$k_{obs} = k_o + k_{H^+}[H^+] + k_{HA}[HA] \qquad (2)$$

By suitably varying the conditions under which the reaction is carried out the terms k_o, k_{H^+} and k_{HA} can be evaluated. Thus,

by varying [HA] at constant pH it is possible to obtain a series of k_{obs} values which when plotted against [HA] give a straight line whose gradient equals k_{HA}. It is then possible to vary $[H^+]$ and obtain another set of k_{obs} values. A graph of $(k_{obs}-k_{HA}[HA])$ versus $[H^+]$ will be linear with a slope equal to k_{H^+} and an intercept equal to k_o.

Appendix 2

Swinbourne-Kezdy method for the determination of a first-order rate constant (k)

It is often impracticable to measure the initial concentration or concentration after 'infinite' time of a reactant during a kinetic study and so outlined below is a method which overcomes these problems.

Consider a first-order reaction of which observations $(x_0, x_1, x_2, \ldots x_n \ldots x_\infty)$ are taken at times $(t_0, t_1, t_2 \ldots t_n \ldots t_\infty)$.

For a reading (x_n) taken at (t_n)

$$(x_\infty - x_n) = (x_\infty - x_0) \exp(-kt_n) \qquad (1)$$

Now consider a second series of observations $(x_0, x_1', x_2', \ldots x_n' \ldots x_\infty)$ taken at times $(t_0, t_1 + \Delta t, t_2 + \Delta t, \ldots t_n + \Delta t \ldots t_\infty)$ where Δt is a small, constant time interval.

For a reading (x_n') taken at time $(t_n + \Delta t)$

$$(x_\infty - x_n') = (x_\infty - x_0) \exp[-k(t_n + \Delta t)] \qquad (2)$$

Dividing (1) by (2) and rearranging gives

$$(x_\infty - x_n) \exp(kt_n) = (x_\infty - x_n') \exp[k(t_n + \Delta t)] \qquad (3)$$

Therefore,

$$x_n = x_\infty [1 - \exp(k\Delta t)] + x_n' \exp(k\Delta t)$$

A straight line is obtained when plotting the observed readings in the first series (x_n) against the corresponding readings in the second series (x_n'), and the rate constant (k) of the reaction can be evaluated from the log of the slope of the line.

For $t = \infty$, $x_n = x_n' = x_\infty$ and therefore x_∞ is the point on the line at which x_n and x_n' are equal. Also, if the time of commencement of the reaction is known it is possible to

extrapolate back along the line and find the corresponding value of x_0.

The following features should, however, be noted when employing this method :

(1) readings taken towards the end of the reaction are 'telescoped' on the graph and, therefore, are weighted less than earlier readings.

(2) the data should be recorded over a period of time greater than $t_{\frac{1}{2}}$ (the half-life of the reaction) and preferably greater than twice this period. The time interval Δt should be in the range $0.5\ t_{\frac{1}{2}} \rightarrow t_{\frac{1}{2}}$.

(3) the method is relatively insensitive to deviations from the strict first-order law, so an independent check of this is advisable.

Appendix 3

Linearised rate equations and relaxation times

It is found that the rate of disappearance of a (small) difference between the actual and equilibrium concentrations of a component in a fast reaction is proportional to this difference itself. The reciprocal of the proportionality factor has the dimensions of time and is called the relaxation time (τ).

For a single-step reaction (of arbitrary order), the linearised rate can be generally expressed as

$$dx_i/dt + (1/\tau)x_i = (1/\tau)\bar{x}_i \qquad (1)$$

in which x_i and $\bar{x}_i$ are defined as concentration differences of a reacting species i. $x_i = C_i - C_i^o$ is the difference between the actual concentration C_i and a time-independent reference value C_i^o (ie the concentration belonging to reference external conditions). $\bar{x}_i = \bar{C}_i - C_i^o$ is the corresponding difference between the (possibly time-dependent) equilibrium concentration $\bar{C}_i$ and the reference value C_i^o. Equation (1) is valid only under the conditions

$$\bar{x}_i - x_i \ll C_i \qquad (2)$$

For the example

$$A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C$$

the complete rate equation is given by

$$-dC_A/dt = -dC_B/dt = dC_C/dt = k_1 C_A C_B - k_{-1} C_C \qquad (3)$$

By using the above definitions of x_i and $\bar{x}_i$

$$
\begin{aligned}
C_A &= C_A^o + x_A & \bar{C}_A &= C_A^o + \bar{x}_A \\
C_B &= C_B^o + x_B & \bar{C}_B &= C_B^o + \bar{x}_B \\
C_C &= C_C^o + x_C & \bar{C}_C &= C_C^o + \bar{x}_C \\
x_A &= x_B = -x_C & \bar{x}_A &= \bar{x}_B = -\bar{x}_C
\end{aligned}
\qquad (4)
$$

For small differences $(\bar{x}_i - x_i)$

$$
dC_A/dt = dx_A/dt = k_{-1}(\bar{C}_C + x_C - \bar{x}_C) - k_1(\bar{C}_A + x_A - \bar{x}_A)(\bar{C}_B + x_B - \bar{x}_B) \qquad (5)
$$

In the situation where B is buffered, which is the situation encountered in Chapter 2 (B = DMAB), $x_B \approx 0$.

Equation (5) then reduces to

$$
dx_A/dt = k_{-1}\bar{C}_C + k_{-1}(\bar{x}_A - x_A) - k_1\bar{C}_A\bar{C}_B - k_1(x_A - \bar{x}_A)\bar{C}_B \qquad (6)
$$

$k_{-1}\bar{C}_C = k_1\bar{C}_A\bar{C}_B$ represents the equilibrium condition. Thus,

$$
dx_A/dt + [k_{-1} + (k_1\bar{C}_B)]x_A = [k_{-1} + (k_1\bar{C}_B)]\bar{x}_A \qquad (7)
$$

which is equivalent to equation 1 with

$$
1/\tau = [k_{-1} + k_1\bar{C}_B] \qquad (8)
$$

Since the relaxation time is inversely proportional to the experimentally determined rate constant (k_{obs}), equation (8) can be rewritten as

$$
k_{obs} = k_1\bar{C}_B + k_{-1} \qquad (9)
$$

Appendix 4

Acidity Functions

Since the Arrhenius theory of electrolytic solutions was proposed the normal measure of acidity has been hydrogen-ion concentration or activity. Although these quantities lead to sufficiently accurate results in dilute aqueous solutions, in concentrated solutions or in non-aqueous solvents results calculated using these 'acidity' measurements tend to have sizeable errors. It is, therefore, desirable to have a more universal measurement of acidity which can be applied to a wider range of situations.

Acidity functions, based on the protonation of certain basic indicators, are found to have just such properties. As opposed to measuring some form of hydrogen-ion concentration in solution, these functions measure the tendency of any given solution to donate a proton to a base. This is a particularly useful quantity in chemical kinetics where, in many cases of acid catalysis, the initial process is transfer of a proton from solvent to base.

The protonation of a base can be written as

$$B + H^+ \rightleftharpoons BH^+ \qquad (1)$$

where $K_B = \dfrac{a_{BH^+}}{a_B a_{H^+}}$ (2)

and $K_a = \dfrac{a_{H^+} a_B}{a_{BH^+}}$ (3)

where a represents the activity of the species.

Equation (3) can be written as

$$K_a = \frac{[H^+][B]}{[BH^+]} \quad \frac{f_{H^+}f_B}{f_{BH^+}} \qquad (4)$$

where f represents the activity coefficient of the species.

Therefore,

$$[H^+] = K_a \frac{[BH^+]}{[B]} \quad \frac{f_{BH^+}}{f_{H^+}f_B} \qquad (5)$$

In dilute concentrations the activity coefficients tend to unity and therefore equation (5) can be expressed as

$$[H^+] = \lim_{[H] \to 0} K_a \frac{[BH^+]}{[B]} \qquad (6)$$

Therefore

$$-\log [H^+] = pH = pK_a - \log \frac{[BH^+]}{[B]} \qquad (7)$$

This argument can be extended to concentrated solutions and by analogy the acidity function H_o for a neutral base (B) is defined by

$$H_o = pK_a - \log \frac{[BH^+]}{[B]} \qquad (8)$$

Similarly for singularly charged bases B^+ and B^-

$$H_+ = pK_a - \log \frac{[BH^{++}]}{[B^+]} \qquad (9)$$

and $$H_- = pK_a - \log \frac{[BH]}{[B^-]} \qquad (10)$$

Equation (8) can be written as

$$H_o = -\log \frac{a_{H^+} f_B}{f_{BH^+}} \qquad (11)$$

It is clear from this equation that the value of H_o will be independent of the base used only if the ratio of f_B to f_{BH^+} has the same value for all bases in any given medium. Although this is not generally true, it is for aqueous solutions of the four

strong acids, sulphuric, perchloric, hydrochloric and nitric. From equation (11) it is also obvious that for dilute solutions, where f_B and f_{BH^+} tend to unity, that H_o is exactly equivalent to pH. A convenient function related to H_o is h_o, defined by the equation

$$H_o = -\log h_o \tag{12}$$

To measure H_o both the ratio $[BH^+]/[B]$ and the pK_a must be determined. The ratio $[BH^+]/[B]$ is called the indicator ratio (I). A simple basic indicator is merely a monoacidic base whose ionisation is accompanied by a change in light absorption normally in the visible region. This change in absorption provides a means of determining the indicator ratio.

Thus

$$\frac{[BH]}{[B]} = I = \frac{\varepsilon^{\lambda}_{B} - \varepsilon^{\lambda}}{\varepsilon^{\lambda} - \varepsilon^{\lambda}_{BH^+}} \tag{13}$$

where $\varepsilon^{\lambda}_{B}$ is the extinction coefficient at a given wavelength of a solution containing the base entirely in the non-protonated form. $\varepsilon^{\lambda}_{BH}$ is the extinction coefficient of a solution containing the base entirely in the protonated form and ε^{λ} is the extinction coefficient of a solution in which the base is partly protonated.

It should be noted that the base concentration should be sufficiently low so that the Beer-Lambert law is applicable and to ensure that it does not change the acid concentration significantly.

From equation (4)

$$-\log K_a = pK_a = \log \frac{[BH^+]}{[B][H]} + \log \frac{f_{BH^+}}{f_B f_{H^+}} \tag{14}$$

Since the indicator concentration is always extremely low equation (14) becomes equivalent to

$$pK_a = \lim_{[A] \to 0} \left[\log \left(\frac{[BH^+]}{[B]} \right) - \log [H^+] \right] \qquad (15)$$

where [A] is the acid concentration.

For a strong acid $[H^+]$ is found from the relation

$$[A] = [H^+] + [BH^+] \qquad (16)$$

Thus, for a dilute aqueous solution of a strong acid the pK_a value can be determined from the observed values of [A] and I.

For weak bases this type of direct measurement of pK_a values cannot be carried out and a step-wise approach must be used using two different bases (B and C) in the same solution. Thus

$$pK_{CH^+} - pK_{BH^+} = \log \left(\frac{[CH^+]}{[C]} \right) - \log \left(\frac{[BH^+]}{[B]} \right) - \log \left(\frac{f_C f_{BH^+}}{f_{CH} f_B} \right) \qquad (17)$$

For bases which are structurally similar it is found that the last term in equation (17) can be neglected in aqueous solution. Therefore, provided B and C have ionisation constants sufficiently close together to enable both indicator ratios to be measured with good precision in the same solution, the differences in the pK_a values can be determined using equation (17). This method can be extended to include any other indicator (D) whose ionisation overlaps that of either B or C. Now, if any of the pK_a values of these indicators can be measured directly by the method described previously then the pK_a values of the other indicators can be found.

The initial H_o values were determined using substituted anilines as the bases but this has subsequently been extended to a wide range of bases with a corresponding range of different acidity scales being generated.

Appendix 5

The derivation of thermodynamic functions from kinetic data

From the Arrhenius equation,

$$k = A \exp^{-E/RT}$$

(where A is the exponential factor in units of k), a plot of log k versus $^{1}/_{T}$ yields E (where all temperature values are in units of Kelvin), the Arrhenius energy of activation (cal mol^{-1}), from the relationship

$$E = -4.576 \times \text{slope}.$$

Similarly, the enthalpy of activation (cal mol^{-1}) is found from

$$\Delta H^{\#} = E-RT$$

where T is taken as a mean value over the range studied. The entropy of activation, $\Delta S^{\#}$ (cal mol^{-1} $degree^{-1}$ or entropy units), may be determined from the relationship,

$$\Delta S^{\#} = 4.576 \log ({}^{k}/_{T}) + ({}^{E}/_{T}) - 49.22$$

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